Received: 18 July 2024 | Revised: 4 September 2024 | Accepted: 19 September 2024

DOI: 10.1002/jha2.1029

eJHaem

British Society for Haematology

RESEARCH ARTICLE

A phase 2 study of frontline pembrolizumab in follicular lymphoma

Carrie Ho[1,2] | Songli Zhu[3] | Ted Gooley[2] | Taranjit S. Gujral[3] | Ryan C. Lynch[1,2] | Christina Poh[1,2] | Mazyar Shadman[1,2] | Stephen D. Smith[1,2] | Yolanda Tseng[2,4] | Ajay K. Gopal[1,2]

[1]Division of Hematology and Oncology, Department of Medicine, University of Washington, Seattle, Washington, USA

[2]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA

[3]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, Washington, USA

[4]Department of Radiation Oncology, University of Washington, Seattle, Washington, USA

Correspondence
Ajay K. Gopal, 825 Eastlake Ave E, Mailstop: LG-650, Seattle, WA 98109, USA.
Email: agopal@uw.edu

Funding information
National Institutes of Health/National Cancer Institute, Grant/Award Numbers: K24CA184039, P30CA015704; National Heart, Lung, and Blood Institute, Grant/Award Number: T32HL007093; Merck Sharpe & Dohme LLC

Abstract

Background: The tumor microenvironment (TME), including infiltrating T-cells, is thought to play a major role in the pathogenesis and prognosis of follicular lymphoma (FL) and may contribute to its widely varied disease course. We hypothesized that programmed death-1 inhibition may be most effective in untreated, immunocompetent FL patients. Thus, we developed a phase 2 study to evaluate the efficacy of pembrolizumab as the initial treatment for indolent B-cell lymphoma.

Methods: Adults with FL or marginal zone lymphoma and an indication for treatment were eligible. Patients received pembrolizumab 200 mg IV in 21-day cycles for up to 18 cycles, until progression or unacceptable toxicity. Early response assessment was obtained after cycle 3 with computed tomography (CT), and a fluorodeoxyglucose (FDG)-positron emission tomography-computed tomography (PET-CT) was obtained after cycle 6 to determine candidacy for continuation in the study. Immunosecretome profiling was performed at baseline and on cycle 2 day 1.

Results: Nine patients with FL were enrolled between February 2019 and April 2021, including eight (89%) with advanced stage, seven (78%) with intermediate/high Follicular Lymphoma International Prognostic Index, and six (67%) with high-tumor burden by Groupe d'Etude des Lymphomes Folliculaires. The best overall response rate by FDG PET-CT was 33% (three partial metabolic responses). Three patients (33%) had stable disease, and three (33%) had progressive disease (including one patient who only had a follow-up CT). By CT four (44%) experienced a reduction in target lesions, but all were less than partial responses. Grade 3 or higher immune-related adverse events (IRAEs) were seen in two (22%) patients, both with transaminitis and one of whom had concurrent hypophysitis. Another patient had grade 1 pneumonitis, requiring treatment with steroids. No associations between the immunosecretome profile and clinical outcomes could be detected.

Conclusion: Frontline pembrolizumab for FL is associated with limited responses and a clinically significant rate of IRAEs. Alternative strategies for targeting the TME in FL should be explored.

KEYWORDS
follicular lymphoma, immunotherapy, PD-L1

1 | BACKGROUND

Follicular lymphoma (FL) accounts for approximately 35% of non-Hodgkin lymphomas (NHLs), and CD20 antibody-based immunochemotherapy represents the standard of care for most patients with advanced-stage disease. However, cytotoxic chemotherapy is associated with myelosuppression, cumulative toxicity, and successively shorter remissions. Recent trials demonstrate that single-agent rituximab is highly effective in low-tumor burden FL and that advanced-stage symptomatic disease may be successfully treated with lenalidomide-rituximab in most patients [1, 2]. Novel therapies that target the tumor microenvironment may provide additional non-cytotoxic frontline treatment options for patients with FL.

Studies have shown that the host immune response impacts outcomes in FL [3]. Preclinical studies have demonstrated that the tumor microenvironment of FL, which is characterized by an abundance of nonmalignant cells, has prognostic significance. High numbers of T regulatory cells and specific gene expression signatures of tumor-infiltrating immune cells have been associated with improved survival [4–7]. Despite this, harnessing antitumor immunity for therapeutic benefit has proven challenging in FL. The programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) pathway plays a role in modulating the activity of T cells and in facilitating tumor immune escape. While PD-L1 expression on FL tumor cells is uncommon, characteristics of the non-malignant local tumor microenvironment, such as the presence of PD-1 positive tumor-infiltrating lymphocytes (TILs), have prognostic significance, albeit with inconsistent results across various studies [8–10].

Early phase studies of PD-1/PD-L1 inhibition in relapsed/refractory (R/R) FL have yielded inconsistent results. Despite an early study of nivolumab in FL patients showing an overall response rate (ORR) of 40% (4/10), a larger trial (Checkmate 140) demonstrated responses in only 4% of patients, all of whom had received at least two prior lines of therapy that included a CD20 antibody or an alkylating agent [11, 12]. Furthermore, a phase 2 study of pembrolizumab plus rituximab showed an ORR of 67%, with 50% of patients obtaining a complete response (CR) [13]. Concurrent administration of rituximab, however, makes it difficult to discern the relative contribution of pembrolizumab.

PD-1+ TILs have also been observed in marginal zone lymphoma (MZL), and gene expression studies in splenic MZL (sMZL) suggest that approximately 50% of cases exhibit an immune-evasion profile characterized by immune checkpoint activation and T-cell exhaustion [8, 14]. While clinical data on immune checkpoint inhibition in MZL are limited, there is a case report of a patient with sMZL and high PD-L1 expression who achieved a deep molecular response with frontline pembrolizumab [15].

In contrast to the R/R setting, we hypothesized that frontline PD-1 inhibition, prior to successive rounds of lymphodepleting chemotherapy and the emergence of resistant clones, may enhance antitumoral immune responses and lead to higher efficacy. Thus, we tested single-agent PD-1 inhibition among treatment-naïve FL and MZL patients to assess the potential efficacy and safety of pembrolizumab and explore biomarkers associated with response and toxicity.

2 | MATERIALS AND METHODS

2.1 | Patients

We conducted an investigator-initiated, open-label, single-center, single-arm phase 2 study of pembrolizumab for untreated indolent B-cell NHL. This study was approved by the Fred Hutchinson Cancer Center Institutional Review Board and performed in accordance with the ethical principles in the Declaration of Helsinki. All patients provided written informed consent. The trial was registered at www.clinicaltrials.gov (NCT 03498612).

Patients aged 18 years or older with untreated indolent B-cell NHL, including FL and MZL, and an indication for treatment by NCCN guidelines (significant symptoms due to the lymphoma, threatened end-organ function, progressive cytopenias, or steady progression of disease), were eligible. Additional inclusion criteria included having measurable disease (lesion greater than 1.5 cm by computed tomography [CT] or magnetic resonance imaging [MRI]), an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and adequate hematopoietic and organ function (absolute neutrophil count $\geq$ 500/μL, platelets $\geq$ 25,000/μL, hemoglobin $\geq$ 8 g/dL, creatinine $\leq$ 1.5 $\times$ upper limit normal [ULN] or $\geq$ 30 mL/min, total bilirubin $\leq$ 1.5 ULN, AST and ALT $\leq$ 2.5 $\times$ ULN, INR $\leq$ 1.5 unless on anticoagulant therapy). Notable exclusion criteria included a history of immunodeficiency, having received steroids or any immunosuppressive agent within 7 days of treatment start, and active autoimmune disease requiring systemic treatment within the past 2 years.

2.2 | Study design

In the window phase, pembrolizumab was administered at 200 mg IV in 21-day cycles for up to 6 cycles. Early tumor assessment was performed with CT imaging after cycle 3. Fluorodeoxyglucose (FDG)-positron emission tomography-CT (PET-CT) imaging with assessment by Lugano criteria was performed after cycle 6 to determine candidacy for continuation of study [16]. For those tolerating the drug and showing an objective response after cycle 6, pembrolizumab could be continued for up to an additional 12 cycles, with response assessments prior to cycles 11 and 15. Those who had disease progression or stable disease (PD or SD) after the initial six cycles could continue treatment in the window phase, with restaging after another three cycles to assess for pseudo-progression or a delayed response. Patients were taken off the study if disease progression was seen after the additional three cycles. An end-of-treatment evaluation was performed within 30 days of the last dose of pembrolizumab or prior to the next line of therapy, whichever came first. Adverse events (AEs) were collected and graded per Common Terminology Criteria for Adverse Events version 4.0.

2.3 | Endpoints and statistical considerations

The primary endpoint for this study was OR (CR and partial response [PR]) at the end of the 6-cycle window treatment period. Secondary endpoints included event-free survival (EFS), time to next therapy (TTNT), and safety. EFS was defined as the time from the start of pembrolizumab to progression, the start of the next line of lymphoma treatment, or death.

A benchmark ORR of 25% was used, based on early data of immune checkpoint inhibition in R/R FL [17]. The trial followed a Simon two-stage minimax design, with an assumed-true ORR of 50% associated with pembrolizumab [18]. In the first stage of the Simon design, if there are four or fewer responses among the first 16 patients (25% or less) enrolled, consideration would be given to terminating the study due to lack of efficacy. If there are five or more responses among the first 16 patients, an additional 17 patients (for a total of 33) will be enrolled. Twelve or fewer (36% or less) responses among 33 would not allow the null hypothesis of an ORR of 25% to be rejected. This design has 90% power and a type I error rate of 0.045, and the expected sample size under the null hypothesis is 22 patients. Additionally, if there are zero responses amongst the first six patients enrolled within a particular histology, accrual of that histology would be stopped. A stopping rule for safety was included, such that the study would be suspended if the observed rate of infusion or immune-related AEs of grade 3 or higher was 12% or higher. Operationally, this would occur if three out of the first 11 or fewer, five out of the first 22 or fewer, or six out of the first 33 or fewer patients have grade 3 or higher immune-related or infusion-related AEs.

Descriptive statistics were used to summarize clinical and demographic information. The Kaplan-Meier method was used to estimate time-to-event outcomes in months with a corresponding 95% confidence interval (CI). Statistical software R version 2023.12.1+402 was used for analyses.

2.4 | Correlative studies

Secreted cytokines, chemokine, and growth factors were measured either by Luminex or nano ELISA (nELISA) as previously described, in available plasma or peripheral blood mononuclear cells (PBMCs) from six patients at baseline pre-treatment and prior to treatment on cycle 2 day 1 (C2D1) [19–21]. Briefly, plasma samples from four cases (patients 6–9) were collected and 71 secreted factors were measured by Luminex technology [20]. For two patients (patients 4 and 5), who did not have available plasma samples, PBMCs were cultured in RPMI media supplemented with 10% fetal bovine serum. After 24 h, the conditioned media was collected and 187 secreted factors were measured using nELISA [21]. For each measurement, at least 2–3 technical replicates were used. The data were normalized to the baseline control, and fold changes were determined for each case. Correlations were explored both with clinical response and immune-related toxicity, but given limited sample sizes, the results were descriptive. An immune-multiple reaction monitoring mass spectrometry (MRM-MS)-based proteomic assay representing 43 immunomodulatory proteins, was performed on plasma samples from four patients at baseline pre-treatment as well as on C2D1 [22].

3 | RESULTS

3.1 | Patient demographics

A total of nine patients (all with FL) were enrolled in the trial from February 2019 to April 2021 (Table 1). The study was terminated early due to slow accrual despite no screen failures. The median age was 51 years (range, 39–70), three (33%) were female, and all were white. Eight (89%) had stage III or IV disease. All had grade 1–2 FL. The Follicular Lymphoma International Prognostic Index (FLIPI) score was low, intermediate, and high risk in two (22%), five (55%), and two (22%) patients, respectively. No patients had bulky disease, defined as the largest diameter lesion $\geq$ 7 cm, but six (67%) had high-tumor burden disease according to Groupe d'Etude des Lymphomes Folliculaires criteria. One (11%) patient had B symptoms. Indication for treatment was significant disease-related symptoms in seven (78%) patients and steady or rapid disease progression in the remaining two (22%) patients.

3.2 | Efficacy

Patient outcomes are depicted in Figures 1 and 2. By PET-CT assessment of metabolic response after six cycles or at the end of treatment, 3/9 (33%) had the best response of PR, 2/9 (22%) SD, 3/9 (33%) PD,

TABLE 1 Baseline patient characteristics. ECOG = Eastern Cooperative Oncology Group. GELF = Groupe d'Etude des Lymphomes Folliculaires Criteria. LDH = lactate dehydrogenase.

Characteristic	*N* = 9
Age, median (range)	51 (39-70)
Female	3 (33%)
Race, White	9 (100%)
Ann Arbor Stage	
2	1 (11%)
3	4 (44%)
4	4 (44%)
Grade 1–2	9 (100%)
FLIPI Risk Score	
0–1 (Low)	2 (22%)
2 (Intermediate)	5 (55%)
≥ 3 (High)	2 (22%)
Bulky disease (≥ 7 cm)	0
Elevated LDH	2 (22%)
B symptoms	1 (11%)
High-tumor burden by GELF	6 (66%)
ECOG	
0	7 (78%)
1	2 (22%)
Indication for treatment	
Symptoms	7 (78%)
Threatened end-organ function	0
Cytopenias	0
Bulky disease	0
Steady or rapid progression	2 (22%)

and one patient did not have a PET/CT performed but was scored as PD based on CT. By CT assessment, four (44%) patients had a reduction in target lesions, although all had less than a partial response (Figure 1). The best response of SD was seen in seven (78%) patients, while two (22%) had progressive disease (PD). The median number of treatment cycles was 4 (range, 1–9). Five (56%) patients discontinued treatment due to PD or SD, and four (44%) patients discontinued due to treatment-related AEs. After a median follow-up among survivors of 50 months (range: 30.7–59.1), the median EFS was 6.3 months (95% CI 3.77–NA) (Figure 2).

Of the nine patients, one was lost to follow-up soon after being taken off the trial, and seven of the remaining eight patients went on to receive the next line of therapy. The median TTNT was 5.4 months (95% CI, 4.73–NA). One patient who came off trial after three cycles of pembrolizumab due to grade 1 thyroiditis and minimal response (SD by CT, PR by PET), has not progressed since and remains off treatment 56 months later. Additionally, one patient with baseline adenopathy in the left axilla, spleen, and abdominal lymph nodes, had SD after receiving nine cycles of pembrolizumab. A biopsy of his left axillary lymph node confirmed residual disease. Given symptomatic left axillary LAD, a decision was to take him off trial, and he subsequently underwent palliative 2 Gy × 2 radiation to the left axilla for local control. PET/CT 3 months following radiation showed a CR, with an abscopal response in areas of disease outside the axilla. He remains without evidence of recurrence, now 56 months out from completion of radiation.

3.3 | Safety

Treatment-related AEs were experienced in all patients (Table 2). All were grade 1 or 2, with the exception of two (22%) patients, who experienced grade 3 transaminitis. Notable immune-related AEs (IRAEs) include grade 1 thyroiditis, which developed after two cycles; grade 2 hypothyroidism, which developed after four cycles; grade 3 transaminitis and grade 2 hypophysitis (manifested by headache, low testosterone, low ACTH, and elevated TSH), which developed after one cycle; grade 3 transaminitis, which developed after three cycles and

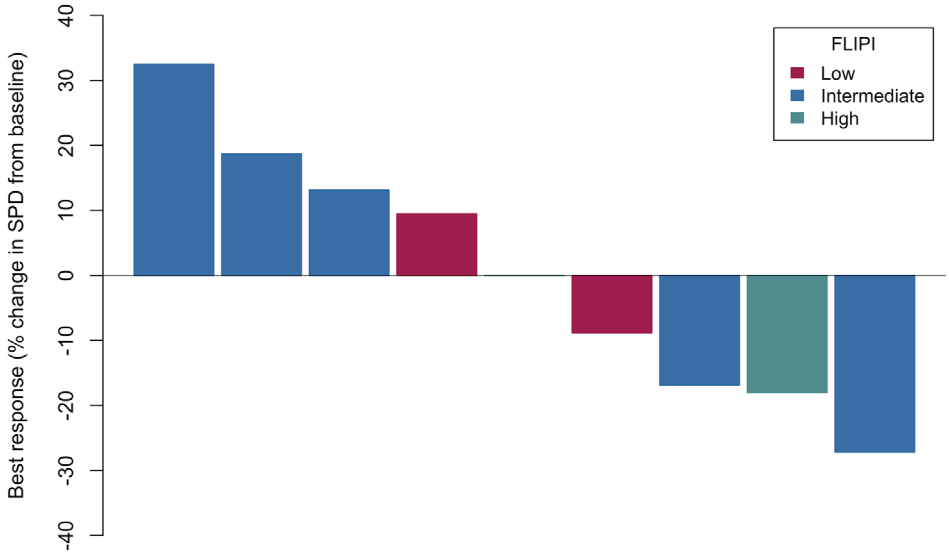

FIGURE 1 Best response, represented by the percentage change from baseline of the sum of product of diameters (SPD) of index lesions.

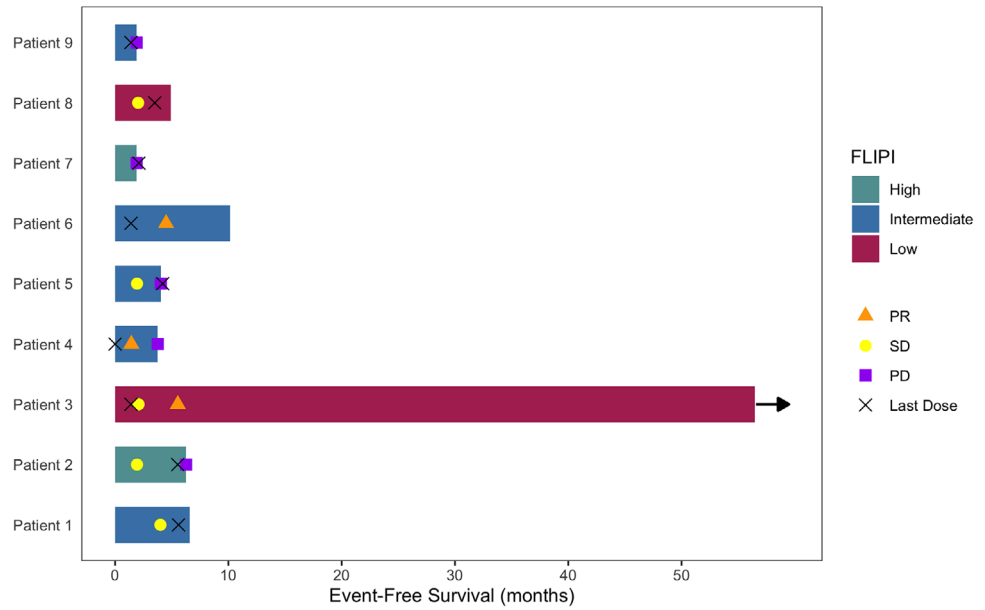

FIGURE 2 Event-free survival (EFS) in months from the start of pembrolizumab treatment. An event was defined as progression, the start of next-line treatment in those who did not progress, or death. In those who did not experience an event, data was censored at the time of the last follow-up. "X" marks the last pembrolizumab dose received. The arrow at the end of the bar in one patient reflects a continued response at the last follow-up despite stopping therapy after 3 cycles of treatment.

TABLE 2 Adverse events graded by Common Terminology Criteria for Adverse Events (CTCAE) 4.0 criteria and attributed as possibly, probably, or likely related to pembrolizumab.

Adverse event, *N* (%)	Grade 1	Grade 2	Grade 3
Pruritus	1 (11%)		
Bilirubin elevation	1 (11%)		
Fatigue	1 (11%)		
Arthralgia	1 (11%)		
Dry eye	1 (11%)		
Dry mouth	1 (11%)		
Hyperthyroidism	1 (11%)		
Headache	1 (11%)	1 (11%)	
Fever	1 (11%)		
Nausea	1 (11%)		
Chills	1 (11%)		
ALT elevation			2 (22%)
AST elevation	1 (11%)	1 (11%)	
Alkaline phosphatase increased	2 (22%)		
Elevated TSH	1 (11%)	1 (11%)	
ACTH decreased	1 (11%)		
Testosterone decreased	1 (11%)		
Hyperglycemia		1 (11%)	
Diarrhea	1 (11%)		
Pneumonitis	1 (11%)		
Constipation	1 (11%)		

grade 1 pneumonitis incidentally noted on imaging after six cycles of treatment.

The two cases of thyroid dysfunction were treated with continued thyroid hormone supplementation. The patient who experienced both hypophysitis and transaminitis was treated with steroids, while the other case of transaminitis was treated with steroids and mycophenolate mofetil. The case of pneumonitis was also treated with steroids. Among the three patients (33%) that received oral corticosteroids, the median duration of steroid treatment was 50 days (range, 5–262 days). No AEs were fatal. Except for the two patients who required ongoing thyroid hormone supplementation, all IRAEs resolved with permanent discontinuation of pembrolizumab, supportive care, and steroids.

3.4 | Correlative studies

Six patients had immunosecretome levels measured at baseline and on C2D1, two from PBMCs, and four from plasma samples (Figures S1 and S2). Two of the six patients had a PR, two had SD, and two had PD. Given our limited sample size, we were not able to perform any formal statistical analyses but amongst the four patients who had immunosecretome analysis performed on plasma samples, we explored associations between baseline or C2D1 immunosecretome levels, fold-change between baseline and C2D1, and clinical responses (Figure 3). We did not identify any meaningful differences between the response groups with regard to baseline immunosecretome levels. However, there were five cytokines/chemokines that were substantially reduced (> 2-fold change from baseline to C2D1) in the patient who achieved a PR compared to other response groups (SD and PD): interleukin (IL)-6,

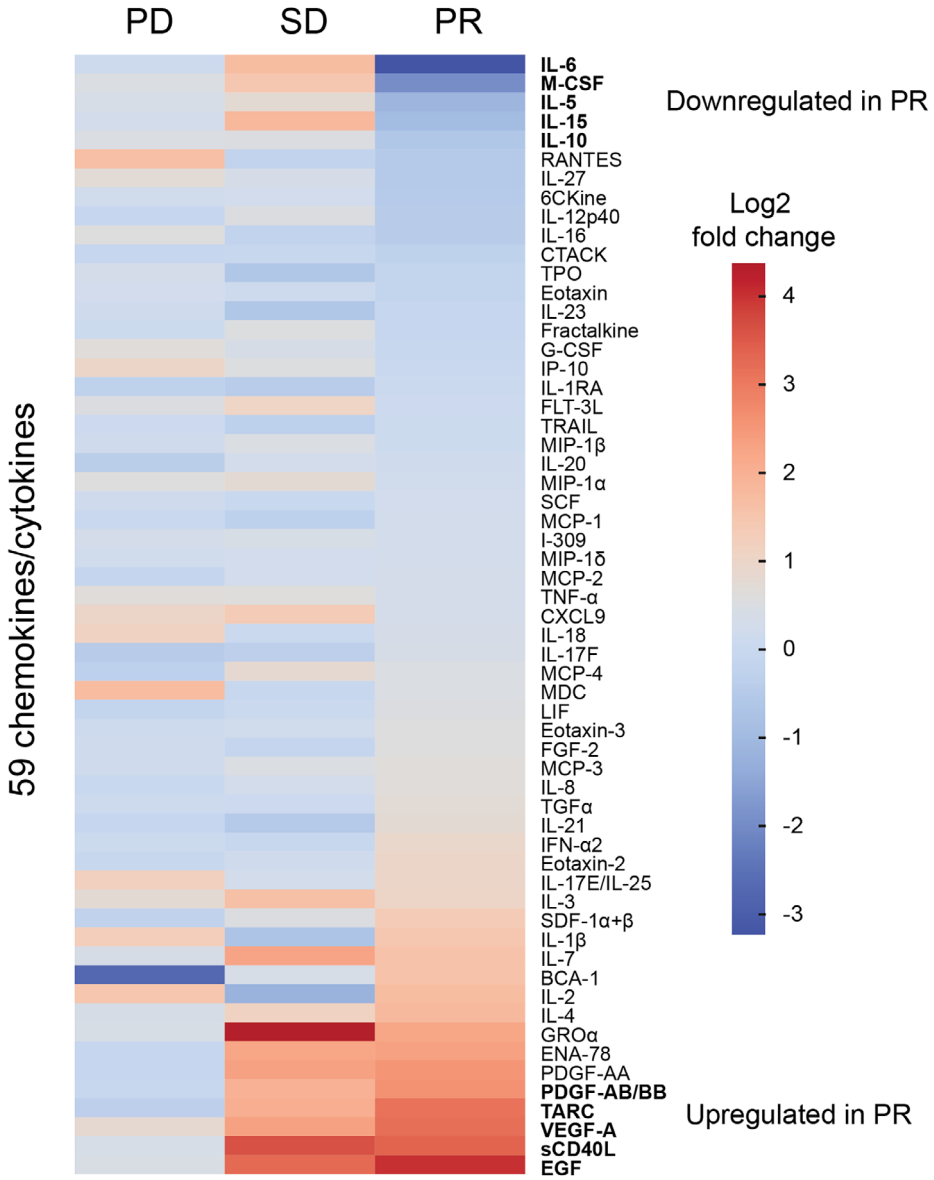

FIGURE 3 Heatmap of Log2-fold changes in cytokine expression between pre-treatment and C2D1, grouped by response categories.

IL-5, IL-10, IL-15, and macrophage colony-stimulating factor (M-CSF) (Figure S3). In the patient who achieved a PR, the five cytokines that showed the most substantial increases compared to baseline levels were PDGF-AA/BB, TARC, VEGF-A, and sCD40L (Figure S4). These observed differences in cytokine changes were numerical but not statistically significant. As all six patients tested experienced an IRAE, we were unable to identify any predictors of IRAEs.

An immuno-MRM assay was performed on four patients, which demonstrated increases in the proteins PDCD1LG2, CD33, CD74, TNFRSF14, IL6R, MPO, LGALS9, and FOXO1 from baseline to C2D1, as previously reported [22].

4 | DISCUSSION

Although hindered by small sample size and early study termination due to slow accrual, our study found that pembrolizumab monotherapy in frontline FL is associated with limited clinical responses and a relatively high rate of IRAEs. When compared to the current standard of care for frontline FL, which is associated with response rates of 85–90%, our data confirms that PD-1 inhibition in FL results in a low response rate, even when applied in the frontline setting [23, 24].

We posit that the slow accrual for this trial may have been due to patient and provider hesitancy to employ pembrolizumab as a monotherapy in patients who had an indication for treatment, given its limited track record in FL. To improve accrual in future trials of novel agents, strategies such as a "window-of-opportunity" trial design to assess the clinical activity of pembrolizumab monotherapy prior to the addition of standard treatments could be considered [25]. Alternatively, testing novel agents in patients with asymptomatic low-tumor-burden disease, where there is less urgency for treatment, might also be a viable approach.

There has been one other reported study of immune-checkpoint inhibition in frontline FL, incorporating nivolumab priming followed

by nivolumab maintenance versus nivolumab plus rituximab in those without a CR to induction nivolumab; the ORR was 93% (56% CR and 36% PR), although the percentage of responses achieved after nivolumab alone is not reported [26]. While our findings suggest limited activity of PD-1 monotherapy in frontline FL, PD-1 inhibitors may have a synergistic effect with rituximab. This synergy could enhance the antibody-dependent, cell-mediated cytotoxicity effect of natural killer cells induced by rituximab, though more detailed results and longer-term follow-up of this strategy are needed [13, 27].

Preclinical studies in FL have highlighted the prognostic significance of the tumor microenvironment, which is enriched with exhausted T cells expressing a variety of immune checkpoint inhibitors, including PD-1, T-cell immunoglobulin and mucin-domain containing-3, lymphocyte activation gene-3, and T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain [28]. Another potential mechanism of immune escape may be associated with the loss of MHCII expression by FL tumor cells, though MHCII expression varies across patients [7]. These data suggest that multiple mechanisms of tumor immune escape are at play within the TME of patients with FL, and targeting one mechanism alone, as demonstrated in our study with PD-1 inhibition, is likely to be ineffective.

In a disease like FL where most patients can still anticipate a normal life expectancy, maintaining or improving quality of life, while balancing the benefits and toxicities of treatment is crucial. In our study, with a limited sample size of nine patients, 22% of patients had grade 3–4 IRAEs. Additionally, 33% of patients required steroids for the treatment of IRAEs, and 44% of patients discontinued treatment due to AEs. Similarly, in the study of nivolumab in frontline FL, amongst 39 patients enrolled, grade 3 or higher toxicities occurred in 41% during induction, and there were 12 cases (30%) of grade 3–4 IRAEs [26]. Although comparisons of IRAE rates are limited by the small sample sizes in both of these studies of frontline immunotherapy in FL, the rate of IRAEs in treatment-naïve patients appears higher than in those with R/R FL, where grade 3 or 4 IRAEs occurred in 13% of patients receiving pembrolizumab plus rituximab [13]. Additionally, prior studies of pembrolizumab and nivolumab across various tumor types, showed an IRAE rate of 30–40%, with grade 3 or higher IRAEs occurring in 3%–12% of patients [29]. Thus, it is possible that frontline PD-1 inhibition may impose greater risks of IRAEs, perhaps due to a more robust immune response that has not previously been modulated or suppressed by other therapies. Furthermore, this risk may be higher in the setting of PD-1 inhibition monotherapy compared to PD-1 combination therapies with chemotherapy, where effector immune cells are suppressed. While differences in IRAE rates may also be attributed to different disease biology, tumor microenvironments, or chance, it is notable that grade 3 or 4 IRAEs were only seen in 10% of patients in a study of pembrolizumab in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (PR-CHOP) in untreated DLBCL, and in 4%–10% of patients treated with nivolumab or pembrolizumab plus doxorubicin, vinblastine, and dacarbazine for newly diagnosed classic Hodgkin lymphoma [30–32].

In contrast, with our current standard-of-care for untreated FL, grade 3 or higher AEs occurred in approximately 65–75% of patients in the RELEVANCE and GALLIUM trials of frontline rituximab plus lenalidomide or immunochemotherapy, both studies of which treatment-related deaths occurred in 1%–4% of patients [24, 33]. Despite the higher-than-expected IRAE rate observed in our study, the overall AE profile may still be more favorable with immunotherapy approaches. Nevertheless, the need for long-term thyroid hormone replacement and prolonged steroid use can still have a significant impact on quality of life, and the tolerance for these AEs may be lower in treatment-naïve patients with indolent disease.

We performed immunosecretome profiling prior to treatment and at C2D1 to explore potential biomarkers for IRAEs and response. Of particular interest were cytokines that have been reported as potential biomarkers in prior studies of immune-checkpoint inhibitors, including IL-6, IL-1, IL-10, tumor necrosis factor-α, interferon-γ, and transforming growth factor-beta [34–36]. However, the majority of these prior studies were performed in solid tumor patients, and as mechanisms of IRAEs are heterogeneous, their applicability in lymphoma is unknown [37]. Although we sought to explore potential biomarkers in our study by analyzing a broad immunosecretome panel, with our limited sample size, we were unable to identify any signatures definitively predictive of outcomes to PD-1 inhibition in FL patients. Nevertheless, we observed substantial numerical decreases between baseline and C2D1 in levels of IL-6, IL-5, IL-10, IL-15, and M-CSF and numerical increases in PDGF-AA/BB, TARC, VEGF-A, sCD40L, and EGF in the patient who had a partial response compared to those with stable or progressive disease. While larger prospective datasets are needed to corroborate these observations, our data on IL-6 is consistent with current literature, a proinflammatory cytokine that has been demonstrated to be a poor prognostic factor in various cancers, with baseline levels associated with worse outcomes and increases after anti-PD1 therapy being associated with poor clinical response to therapy [38].

5 | CONCLUSION

In summary, our results demonstrate that frontline pembrolizumab monotherapy for follicular lymphoma is associated with limited clinical efficacy and a relatively high rate of IRAEs leading to treatment discontinuation. Nevertheless, the two patients with prolonged disease control in our study, including one with an abscopal response to low-dose radiation therapy following pembrolizumab suggests that manipulation of the immune microenvironment may still hold promise with improved agents and strategies to identify patients most likely to respond.

AUTHOR CONTRIBUTIONS

Carrie Ho performed investigation, formal analysis, data curation, and writing of the original manuscript draft. Songli Zhu and Taranjit S. Gujral performed investigation, formal analysis, data curation, and reviewed and edited the manuscript. Ajay K. Gopal performed study design, investigation, formal analysis, and reviewed and edited the manuscript. Ted Gooley performed formal analysis and reviewed and edited the manuscript. Ryan C. Lynch, Christina Poh, Mazyar Shadman, Stephen D. Smith, and Yolanda Tseng reviewed and edited the manuscript.

ACKNOWLEDGEMENTS

This work was supported in part by a research grant from the Investigator-Initiated Studies Program of Merck Sharp & Dohme LLC. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme LLC. The authors also recognize additional support from the NIH/NCI grant K24CA184039 and NIH/NCI Cancer Center Support grant P30 CA015704, an institutional training grant from NHLBI (T32HL007093), and philanthropic support.

CONFLICT OF INTEREST STATEMENT

Ajay K. Gopal reports consultancy/honoraria from Pfizer, Seagen, Janssen Oncology, SciTek, Compliment Corporation, Millennium, Gilead Sciences, Nurix, Cellectar, Kite/Gilead, Morphosys/Incyte, I-Mab, TG Therapeutics, Pfizer, ADC therapeutics, Amgen, Actinium Pharmaceuticals, Takeda, Epizyme, and Merck; research funding from Merck, Bristol-Myers Squibb, Gilead Sciences, Seagen, Teva, Pfizer, Janssen Oncology, Millennium, IgM, I-Mab, Takeda, BeiGene, and AstraZeneca.

Christina Poh reports research funding from Incyte MorphoSys, Dren Bio, and Astex, and consulting fees from Acrotech and Seagen.

Ryan C. Lynch reports receiving research funding from TG Therapeutics, Incyte, Bayer, Cyteir, Genentech, SeaGen, Rapt, and Merck; and consultancy fees and/or honoraria from SeaGen, Foresight Diagnostics, Abbvie, Janssen, and Merck.

Mazyar Shadman reports consulting, advisory Boards, steering committees, or data safety monitoring committees: Abbvie, Genentech, AstraZeneca, Janssen, Beigene, Bristol Myers Squibb, Morphosys/Incyte, Kite Pharma, Eli Lilly, Mustang Bio, Fate therapeutics, Nurix, Merck. Research Funding: Mustang Bio, Genentech, AbbVie, Beigene, AstraZeneca, Genmab, Morphosys/Incyte, Vincerx. Stock options: Koi Biotherapeutics. Employment: Bristol Myers Squibb (spouse).

Stephen D. Smith reports consultancy from BeiGene, Coherus Biosciences (immediate family member), Genentech, and Merck; research funding from Acerta Pharma/AstraZeneca, Ayala Pharmaceuticals (immediate family member), Bristol-Myers Squibb (immediate family member), Bayer, Denovo Biopharma, Genentech, Ignyta (immediate family member), Incyte, Merck, Portola Pharmaceuticals, BeiGene, ADC Therapeutics, Enterome, Kymera, MorphoSys/Incyte, Nanjing, Portola Pharmaceuticals/Alexion Pharmaceuticals, and Viracta Therapeutics.

All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Fred Hutchinson Cancer Center Institutional Review Board and performed in accordance with the ethical principles in the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All patients provided written informed consent prior to enrollment in the study.

CLINICAL TRIAL REGISTRATION

The trial was registered at www.clinicaltrials.gov (NCT03498612).

ORCID

Carrie Ho https://orcid.org/0000-0003-4437-9377
Songli Zhu https://orcid.org/0000-0001-8560-5444

REFERENCES

1. Williams ME, Hong F, Gascoyne RD, Wagner LI, Krauss JC, Habermann TM, et al. Rituximab extended schedule or retreatment trial (RESORT) for low tumor burden non-follicular indolent B-cell non-hodgkin lymphomas: Eastern Cooperative Oncology Group protocol E4402. Br J Haematol. 2016;173(6):867–75. https://doi.org/10.1111/bjh.14007
2. Gravelle P, Burroni B, Péricart S, Rossi C, Bezombes C, Tosolini M, et al. Mechanisms of PD-1/PD-L1 expression and prognostic relevance in non-Hodgkin lymphoma: a summary of immunohistochemical studies. Oncotarget. 2017;8(27):44960–75. doi: 10.18632/oncotarget.16680
3. Byers RJ, Sakhinia E, Joseph P, Glennie C, Hoyland JA, Menasce LP, Radford JA, et al. Clinical quantitation of immune signature in follicular lymphoma by RT-PCR-based gene expression profiling. Blood. 2008;111(9):4764–70. https://doi.org/10.1182/blood-2007-10-115915
4. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351(21):2159–69. https://doi.org/10.1056/NEJMoa041869
5. Carreras J, Lopez-Guillermo A, Roncador G, Villamor N, Colomo L, Martinez A, et al. High numbers of tumor-infiltrating programmed cell death 1-positive regulatory lymphocytes are associated with improved overall survival in follicular lymphoma. J Clin Oncol Off J Am Soc Clin Oncol. 2009;27(9):1470–76. https://doi.org/10.1200/JCO.2008.18.0513
6. Sugimoto T, Watanabe T. Follicular lymphoma: the role of the tumor microenvironment in prognosis. J Clin Exp Hematop JCEH. 2016;56(1):1–19. https://doi.org/10.3960/jslrt.56.1
7. Han G, Deng Q, Marques-Piubelli ML, Dai E, Dang M, Ma MCJ, et al. Follicular lymphoma microenvironment characteristics associated with tumor cell mutations and MHC class II expression. Blood Cancer Discov. 2022;3(5):428–43. https://doi.org/10.1158/2643-3230.BCD-21-0075
8. Muenst S, Hoeller S, Willi N, Dirnhofer S, Tzankov A. Diagnostic and prognostic utility of PD-1 in B cell lymphomas. Dis Markers. 2010;29(1):47–53. https://doi.org/10.3233/DMA-2010-0725
9. Yang ZZ, Grote DM, Ziesmer SC, Xiu B, Novak AJ, Ansell SM. PD-1 expression defines two distinct T-cell sub-populations in follicular lymphoma that differentially impact patient survival. Blood Cancer J. 2015;5(2):e281–e281. https://doi.org/10.1038/bcj.2015.1
10. Xu-Monette ZY, Zhou J, Young KH. PD-1 expression and clinical PD-1 blockade in B-cell lymphomas. Blood. 2018;131(1):68–83. https://doi.org/10.1182/blood-2017-07-740993
11. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(23):2698–704. https://doi.org/10.1200/JCO.2015.65.9789
12. Armand P, Janssens A, Gritti G, Radford J, Timmerman J, Pinto A, et al. Efficacy and safety results from CheckMate 140, a phase 2

study of nivolumab for relapsed/refractory follicular lymphoma. Blood. 2021;137(5):637–45. https://doi.org/10.1182/blood.2019004753
13. Nastoupil LJ, Chin CK, Westin JR, Fowler NH, Samaniego F, Cheng X, et al. Safety and activity of pembrolizumab in combination with rituximab in relapsed or refractory follicular lymphoma. Blood Adv. 2022;6(4):1143–51. https://doi.org/10.1182/bloodadvances.2021006240
14. Bonfiglio F, Bruscaggin A, Guidetti F, Terzi Di Bergamo L, Faderl M, Spina V, et al. Genetic and phenotypic attributes of splenic marginal zone lymphoma. Blood. 2022;139(5):732–47. https://doi.org/10.1182/blood.2021012386
15. Miller PG, Sperling AS, Gibson CJ, Pozdnyakova O, Wong WJ, Manos MP, et al. A deep molecular response of splenic marginal zone lymphoma to front-line checkpoint blockade. Haematologica. 2020;106(2):651–54. https://doi.org/10.3324/haematol.2020.258426
16. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol Off J Am Soc Clin Oncol. 2014;32(27):3059–68. https://doi.org/10.1200/JCO.2013.54.8800
17. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Preliminary results of a phase I study of nivolumab (BMS-936558) in patients with relapsed or refractory lymphoid malignancies. Blood. 2014;124(21):291. https://doi.org/10.1182/blood.V124.21.291.291
18. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989;10(1):1–10. https://doi.org/10.1016/0197-2456(89)90015-9
19. Chan M, Kang Y, Osborne S, Zager M, Gujral TS. A kinase to cytokine explorer to identify molecular regulators and potential therapeutic opportunities. eLife. 2024;12:RP91472. https://doi.org/10.7554/eLife.91472
20. Chan M, Vijay S, McNevin J, McElrath MJ, Holland EC, Gujral TS. Machine learning identifies molecular regulators and therapeutics for targeting SARS-CoV2-induced cytokine release. Mol Syst Biol. 2021;17(9):e10426. doi: 10.15252/msb.202110426
21. Dagher M, Ongo G, Robichaud N, Kong J, Rho W, Teahulos I, et al. nELISA: A high-throughput, high-plex platform enables quantitative profiling of the secretome. bioRxiv. 2023:2023.04.17.535914. https://doi.org/10.1101/2023.04.17.535914
22. Whiteaker JR, Zhao L, Schoenherr RM, Huang D, Lundeen RA, Voytovich U, et al. A multiplexed assay for quantifying immunomodulatory proteins supports correlative studies in immunotherapy clinical trials. Front Oncol. 2023;13:1168710. https://doi.org/10.3389/fonc.2023.1168710
23. Rummel MJ, Niederle N, Maschmeyer G, Banat GA, Von Grünhagen U, Losem C, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. The Lancet. 2013;381(9873):1203–10. https://doi.org/10.1016/S0140-6736(12)61763-2
24. Morschhauser F, Fowler NH, Feugier P, Bouabdallah R, Tilly H, Palomba ML, et al. Rituximab plus lenalidomide in advanced untreated follicular lymphoma. N Engl J Med. 2018;379(10):934–47. https://doi.org/10.1056/NEJMoa1805104
25. Glimelius B, Lahn M. Window-of-opportunity trials to evaluate clinical activity of new molecular entities in oncology. Ann Oncol. 2011;22(8):1717–25. https://doi.org/10.1093/annonc/mdq622
26. Hawkes EA, Lee ST, Chong G, Gilbertson M, Grigg A, Churilov L, et al. Immune priming with nivolumab followed by nivolumab and rituximab in first-line treatment of follicular lymphoma: the phase 2 1st FLOR study. J Clin Oncol. 2021;39(15_suppl):7560–7560. https://doi.org/10.1200/JCO.2021.39.15_suppl.7560
27. Quatrini L, Mariotti FR, Munari E, Tumino N, Vacca P, Moretta L. The immune checkpoint PD-1 in natural killer cells: expression, function and targeting in tumour immunotherapy. Cancers. 2020;12(11):3285. https://doi.org/10.3390/cancers12113285
28. Laurent C, Dietrich S, Tarte K. Cell cross talk within the lymphoma tumor microenvironment: follicular lymphoma as a paradigm. Blood. 2024;143(12):1080–90. https://doi.org/10.1182/blood.2023021000
29. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current diagnosis and management of immune-related adverse events (irAEs) induced by immune checkpoint inhibitor therapy. Front Pharmacol. 2017;8:49. https://doi.org/10.3389/fphar.2017.00049
30. Ho C, Gopal AK, Till BG, Shadman M, Lynch RC, Cowan AJ, et al. Pembrolizumab with R-CHOP in previously untreated DLBCL: sustained, high efficacy, and safety with long-term follow-up. Clin Lymphoma Myeloma Leuk. 2024;24(2):e33–e39.e1. https://doi.org/10.1016/j.clml.2023.10.002
31. Ramchandren R, Domingo-Domènech E, Rueda A, Trněný M, Feldman TA, Lee HJu, et al. Nivolumab for newly diagnosed advanced-stage classic Hodgkin lymphoma: safety and efficacy in the phase II checkMate 205 study. J Clin Oncol. 2019;37(23):1997–2007. https://doi.org/10.1200/JCO.19.00315
32. Lynch RC, Ujjani CS, Poh C, Warren EH, Smith SD, Shadman M, et al. Concurrent pembrolizumab with AVD for untreated classic Hodgkin lymphoma. Blood. 2023;141(21):2576–86. https://doi.org/10.1182/blood.2022019254
33. Marcus R, Davies A, Ando K, Klapper W, Opat S, Owen C, et al. Obinutuzumab for the first-line treatment of follicular lymphoma. N Engl J Med. 2017;377(14):1331–44. https://doi.org/10.1056/NEJMoa1614598
34. Les I, Martínez M, Pérez-Francisco I, Cabero M, Teijeira L, Arrazubi V, et al. Predictive biomarkers for checkpoint inhibitor immune-related adverse events. Cancers. 2023;15(5):1629. https://doi.org/10.3390/cancers15051629
35. Kang DaH, Park C-K, Chung C, Oh In-J, Kim Y-C, Park D, et al. Baseline Serum Interleukin-6 levels predict the response of patients with advanced non-small cell lung cancer to PD-1/PD-L1 inhibitors. Immune Netw. 2020;20(3):e27. https://doi.org/10.4110/in.2020.20.e27
36. Chennamadhavuni A, Abushahin L, Jin N, Presley CJ, Manne A. Risk factors and biomarkers for immune-related adverse events: a practical guide to identifying high-risk patients and rechallenging immune checkpoint inhibitors. Front Immunol. 2022;13:779691. https://doi.org/10.3389/fimmu.2022.779691
37. Morad G, Helmink BA, Sharma P, Wargo JA. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. Cell. 2021;184(21):5309–37. https://doi.org/10.1016/j.cell.2021.09.020
38. Tsukamoto H, Fujieda K, Miyashita A, Fukushima S, Ikeda T, Kubo Y, et al. Combined blockade of IL6 and PD-1/PD-L1 signaling abrogates mutual regulation of their immunosuppressive effects in the tumor microenvironment. Cancer Res. 2018;78(17):5011–22. https://doi.org/10.1158/0008-5472.CAN-18-0118

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: Ho C, Zhu S, Gooley T, Gujral TS, Lynch RC, Poh C, et al. A phase 2 study of frontline pembrolizumab in follicular lymphoma. eJHaem. 2024;5:1173–81. https://doi.org/10.1002/jha2.1029