Nivolumab for Relapsed/Refractory Diffuse Large B-Cell Lymphoma in Patients Ineligible for or Having Failed Autologous Transplantation: A Single-Arm, Phase II Study

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PURPOSE Treatment options are limited for patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL). Tumor cells can exploit the programmed death-1 checkpoint pathway to evade immune surveillance. In the current study, we evaluated the efficacy and safety of programmed death-1 blockade by nivolumab in patients with relapsed/refractory DLBCL.

METHODS In this phase II, open-label study, patients with relapsed/refractory DLBCL who were ineligible for autologous hematopoietic cell transplantation (auto-HCT) or who had experienced failure with auto-HCT received nivolumab 3 mg/kg every 2 weeks. We assessed the efficacy and safety of nivolumab as well as genetic alterations of 9p24.1.

RESULTS Among 121 treated patients, patients in the auto-HCT–failed cohort (n = 87) received a median of four nivolumab doses and a median of three doses were administered to those in the auto-HCT–ineligible cohort (n = 34). At a median follow-up of 9 months in the auto-HCT–failed cohort and 6 months in the auto-HCT–ineligible cohort, independently assessed objective response rates were 10% and 3%, and median durations of response were 11 and 8 months, respectively. Median progression-free survival and overall survival were 1.9 and 12.2 months in the auto-HCT–failed cohort and 1.4 and 5.8 months in the auto-HCT–ineligible cohort respectively. All three patients with complete remission—3% of the auto-HCT–failed cohort—had durable response (11 or more, 14 or more, and 17 months). Treatment-related grade 3 and 4 adverse events were reported in 24% of patients. The most common were neutropenia (4%), thrombocytopenia (3%), and increased lipase (3%). Of all evaluable samples for 9p24.1 analysis, 16% exhibited low-level copy gain and 3% had amplification.

CONCLUSION Nivolumab monotherapy is associated with a favorable safety profile but a low overall response rate among patients with DLBCL who are ineligible for auto-HCT or who experienced failure with auto-HCT. Genetic alterations of 9p24.1 are infrequent in DLBCL.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma worldwide.[1] With standard front-line therapy, approximately two thirds of adult patients achieve long-term remission and others who experience chemosensitive relapse may benefit from autologous hematopoietic cell transplantation (auto-HCT).[2,3] However, patients with refractory DLBCL or those who are unsuitable for or who have experienced relapse after auto-HCT have limited treatment options,[4] with a median survival of only 6 to 10 months from progression.[3,5] Early results of chimeric antigen receptor T-cell therapy in small numbers of selected patients with refractory DLBCL

are promising, but the technology is costly and long-term data are not available.[6] Accessible treatments that provide durable responses and improved outcomes are needed in this setting.

Programmed death-1 (PD-1) and its ligands PD-L1/PD-L2 are immune checkpoints that, in healthy populations, downregulate immune response and are crucial for maintaining self-tolerance and preventing autoimmunity.[7,8] Genes that encode PD-L1/PD-L2 are located on chromosome 9p24.1.[9] Genetic alterations at the locus lead to ligand overexpression, which is common in Hodgkin lymphoma (HL) but are yet to be fully characterized in DLBCL.[10-12] Overexpression of PD-L1 by tumor cells and on tumor-infiltrating

nonmalignant cells in the tumor microenvironment has the potential to interact with PD-1–expressing T cells and B cells, which results in the inhibition of antitumor immune response.[7,8] Increased PD-L1 expression has been found in certain defined subtypes of large B-cell lymphoma (LBCL), such as primary mediastinal B-cell lymphoma and Epstein-Barr virus (EBV)–positive and select non–germinal center cell DLBCLs, and is associated with inferior overall survival (OS).[8,10,12,13] Thus, blockade of the PD-1/PD-L1 pathway may have the potential to exert antitumor effects in certain subsets of DLBCL.

Nivolumab is a fully human immunoglobulin G4 anti–PD-1 monoclonal antibody that blocks tumor cell signaling via the PD-1 pathway, which releases T cells from the inhibitory effects of tumor cells and restores T-cell–mediated anti-tumor immune responses.[14] In clinical trials, nivolumab monotherapy has demonstrated activity in solid tumors, including melanoma, non–small-cell lung cancer, renal cell cancer, and bladder cancer, among others,[15-18] and in relapsed/refractory classic HL (cHL).[19,20] In a phase I dose-escalation study of nivolumab monotherapy in patients with relapsed/refractory hematologic malignancies, four of 11 patients with DLBCL demonstrated objective responses.[21] These preliminary results led to this phase II study, which evaluated the efficacy and safety of nivolumab mono-therapy in patients with relapsed/refractory DLBCL after auto-HCT or who were not candidates for auto-HCT.

METHODS

Study Design and Patients

This was a multicenter, single-arm, open-label, phase II study conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and approved by the institutional review board and independent ethics committee. All patients provided written informed consent before trial enrollment.

Eligibility criteria included age 18 years or older and Eastern Cooperative Oncology Group performance status of 0 or 1. Patients had de novo DLBCL or transformed lymphoma (confirmed by biopsy before initiation of the study drug) that had either relapsed after high-dose conditioning chemo-therapy and auto-HCT or was relapsed/refractory after two or more prior multiagent chemotherapy regimens if auto-HCT ineligible. Key exclusion criteria included prior therapy with any an antibody or drug specifically targeting T-cell costimulation or checkpoint pathways, prior allogeneic HCT, known CNS lymphoma, and history of interstitial lung disease.

Treatment

Patients received nivolumab 3 mg/kg intravenously over 60 minutes every 2 weeks until disease progression, un-acceptable toxicity, or withdrawal from the study. Dose delays were permitted for drug-related adverse events

(AEs), but dose escalations or reductions were not. Treatment beyond investigator-assessed disease progres-sion was permitted in patients who did not experience rapid disease progression and who had investigator-determined clinical benefit from nivolumab and stable performance status.

Assessments

Efficacy. Patients were evaluated for tumor response according to the 2007 International Working Group re-sponse criteria for malignant lymphoma[22] using spiral computed tomography/magnetic resonance imaging. Evaluation was performed at baseline, beginning at week 9 and continuing every 8 weeks through the first 8 months of treatment, every 12 weeks during months 9 to 24, and then every 6 months thereafter until disease progression or until the patient initiated a preparative regimen for allogeneic or auto-HCT. Tumor assessments via fluorodeoxyglucose–positron emission tomography were performed at baseline and were required to confirm complete remission (CR). Baseline and all subsequent scans were submitted to an independent radiology review committee (IRC) for assessment.

Safety. Safety evaluations included assessment of AEs, clinical laboratory tests, and physical examination with assessment of Eastern Cooperative Oncology Group per-formance status. Local laboratory assessments were per-formed within 72 hours before dosing. After discontinuation from the study, safety evaluations were scheduled at the first follow-up visit (35 days from the last dose) and the second follow-up (80 days from the first follow-up visit). Patients were then observed every 3 months for ongoing treatment-related AEs and survival.

Biomarkers. In patients with archival tumor biopsies, 9p24.1 genetic alterations were evaluated using a fluo-rescence in situ hybridization (FISH) assay. Probes encompassed CD274 (PD-L1) or PDCD1LG2 (PD-L2) and included a centromeric control. Copy number alterations were defined as previously described[11,23] on the basis of the target:control signal ratio in 50 analyzed tumor cells per DLBCL. Nuclei with a target:control signal ratio of 3 or more: 1 were defined as coamplified for PD-L1 and PD-L2, and those with a signal ratio of more than 1:1 but less than 3:1 were classified as having relative copy gain. Nuclei with a signal ratio of 1:1 were defined as either polysomic if more than two copies per probe or disomic if there were exactly two copies of the target and control probes.[11] For each patient, we noted the percentage and magnitude of 9p24.1 amplification, copy gain, polysomy, and disomy. Patients were classified by the highest observed level of 9p24.1 genetic alteration. Those with 9p24.1 copy gain lacked amplification and those with 9p polysomy lacked 9p24.1 copy gain or amplification. Chromosomal rearrangements that involved PD-L1 or PD-L2 were also detected with this assay.

TABLE 1. Baseline Characteristics and Demographics

Characteristic	Auto-HCT Failed (n = 87)	Auto-HCT Ineligible (n = 34)
Age		
Median, years (range)	62 (24-75)	68 (28-86)
< 65	56 (64)	11 (32)
≥ 65	31 (36)	23 (68)
≥ 75	1 (1)	10 (29)
Male sex	56 (64)	21 (62)
Race		
White	71 (82)	31 (91)
ECOG performance status		
0	35 (40)	9 (26)
1	49 (56)	25 (74)
2*	2 (2)	0
3*	1 (1)	0
Prior lines of therapy		
Median (range)	3 (1-11)	3 (1-7)
≥ 4	21 (24)	10 (29)
Refractory to most recent line of therapy	24 (28)	20 (59)
Transformed disease	5 (6)	1 (3)
Time since diagnosis, median, years (range)	3.3 (< 1-28)	1.5 (< 1-11)
Disease stage at diagnosis		
I	8 (9)	1 (3)
II	12 (14)	6 (18)
III	23 (26)	8 (24)
IV	41 (47)	19 (56)
Not reported	3 (3)	0

NOTE. Data presented as No. (%) unless otherwise indicated. Totals may not equal 100% as a result of rounding.

Abbreviations: auto-HCT, autologous hematopoietic cell transplantation; ECOG, Eastern Cooperative Oncology Group.

*Protocol deviation.

We performed dual immunohistochemical staining of PD-L1 (clone 405.9A11)[24] and PAX5 (24/Pax-5; BD Biosciences, San Jose, CA) to delineate PD-L1 expression on tumor biopsies, as previously described.[19,23] H-score (0 to 300) was calculated by multiplying the percentage of malignant (PAX5[dim+]) cells with PD-L1–positive staining and the average intensity of staining (0 to 3 or more on 50 malignant cells).

Cell-of-origin (COO) assay by gene expression profiling was performed at Bristol-Myers Squibb by targeted expression directly from formalin-fixed, paraffin-embedded sections as previously described.[25] The HTG EdgeSeq DLBCL Cell of Origin Assay comprised probes that targeted 93 genes commonly assessed in lymphomas. Cell Of Origin was assigned using HTG proprietary software (HTG Molecular Diagnostics, Tucson, AZ).

In situ hybridization was performed for EBV-encoded RNA-1 (EBER-1) using formalin-fixed, paraffin-embedded tissue samples. We tested RNA preservation using a nonspecific positive control, and background was assessed with a negative control. Scoring was performed for EBV/EBER if RNA was preserved and score was greater than background. Scores were EBV/EBER positive nuclear staining of any intensity greater than background in tumor cells.

Outcomes

The primary end point was objective response rate (ORR), defined as either partial remission (PR) or CR, as assessed by IRC using the 2007 revised International Working Group criteria. Secondary objectives included duration of response (DOR) on the basis of IRC assessments, IRC-assessed CR rate and duration of CR, IRC-assessed PR rate and duration of PR (DOPR), IRC-assessed progression-free survival (PFS), and investigator-assessed ORR. OS, safety and tolerability, and biomarker assessment were among additional exploratory end points.

Statistical Analyses

Planned sample size was approximately 120 treated patients, divided into two treatment groups on the basis of prior auto-HCT failure (n = 90) or auto-HCT ineligibility (n = 30). The primary analysis population for safety and efficacy was all patients who had received one or more dose of nivolumab. The protocol had an interim analysis that allowed for discontinuation of the study as a result of a lack of efficacy; however, accrual was rapid and the protocol allowed continued enrollment while the interim analysis was performed. This resulted in complete accrual before the interim analysis was concluded. For the auto-HCT–failed cohort, the null hypothesis that the true ORR was 20% or less would be rejected if 25 or more responses were observed in 90 treated patients. For the auto-HCT–ineligible cohort, the sample size of 30 treated patients was determined such that if the observed number of patients with objective response was 10, the true ORR was larger than 17% with 95% confidence.

IRC-assessed ORR, CR, and PR rates, duration of CR, DOPR, and investigator-assessed ORR were summarized for both cohorts separately by binomial response rates and their corresponding two-sided 95% CIs. IRC-assessed DOR, PFS, and OS were analyzed by cohort using the Kaplan-Meier product-limit method with median values and two-sided CI. Survival rate at 6 months was estimated using Kaplan-Meier values from the survival curve. AEs were coded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.[26] All on-study AEs, serious AEs (SAEs), and treatment-related AEs and SAEs were tabulated according to worst grade per National Cancer Institute Common Terminology Criteria for

Adverse Events, version 4.0, by system organ class and Medical Dictionary for Regulatory Activities preferred term.

RESULTS

Patient Baseline Characteristics and Disposition

Patients were enrolled between March 2014 and August 2015 across 45 sites globally (Appendix Fig A1, online only). Of 121 patients treated, 87 had relapsed/refractory disease after auto-HCT and 34 were auto-HCT ineligible. Baseline characteristics of the two groups are listed in Table 1. In general, patients in the auto-HCT–failed group were younger and had better baseline performance status and longer time since diagnosis than did patients who were ineligible for auto-HCT. Patients had received a median of three prior systemic therapies in each cohort. In the auto-HCT–failed cohort, 40 patients (46%) did not receive any additional lines of systemic or radiation therapy between auto-HCT and the initiation of nivolumab. In the auto-HCT–failed group, 40% of patients had germinal center B-cell–like (GCB) subtype and 32% had non-GCB subtype disease, and in the auto-HCT–ineligible group, 56% had GCB and 18% non-GCB subtype disease. Data were missing in the remainder. All but one patient who had evaluable samples were EBV negative (70 of 121 patients), one patient was EBV positive, and the remainder (n = 50) had either nonevaluable data, mixed results (more than one sample), or missing data.

Median number of doses of nivolumab received were four (range, one to 44 doses) in the auto-HCT–failed cohort and three (range, one to 22 doses) in the auto-HCT–ineligible cohort. At the time of analysis, seven patients (8%) in the auto-HCT–failed group were still on treatment, for whom the median duration of treatment was 14 months from the first study dose. No patients in the auto-HCT–ineligible cohort were still on treatment. Disease progression was the primary reason for discontinuing treatment (83%).

Efficacy

At a median follow-up of 9 months (range, 0.1 to 25 months) in the auto-HCT–failed cohort and 6 months (0.2 to 24 months) in the auto-HCT–ineligible cohort, IRC-assessed ORRs were 10% and 3%, respectively (Table 2). Nearly one third of patients (31%) in the auto-HCT–failed cohort had IRC-assessed stable disease or better response (Fig 1A). Of the nine responders in the auto-HCT–failed cohort, CR occurred in three, with durable response and DOR of 11 or more months, 14 or more months, and 17 months at data cutoff. Of these three patients who achieved CR, two were still on treatment and one developed myelodysplastic syndrome unrelated to study drug and subsequently died. The other six patients achieved PR, with a median DOPR of 7 months (95% CI, 3 to 11 months). The one patient with IRC-assessed PR in the auto-HCT–ineligible group had a DOR of 8.3 months. Patients who achieved CR or PR had early responses, with a median time to response of 1.9 months (Fig 1C). Responses were observed in both the GCB subtype (CR in one and PR in four patients) and non-GCB subtype (CR in one and PR in three patients). Among responders, eight were EBV negative, one was not evaluable, and one had missing data (Appendix Table A1, online only). One patient with PR had

TABLE 2. Response to Nivolumab Treatment

Treatment Response	Auto-HCT Failed (n = 87)		Auto-HCT Ineligible (n = 34)	
	IRC	INV	IRC	INV
Overall response rate,* No. (%)	9 (10)	16 (18)	1 (3)	1 (3)
Nonresponders†	78 (90)	71 (82)	33 (97)	33 (97)
Best overall response, No. (%)				
Complete remission	3 (3)	4 (5)	0	0
Partial remission	6 (7)	12 (14)	1 (3)	1 (3)
Stable disease	18 (21)	12 (14)	3 (9)	1 (3)
Progressive disease	41 (47)	43 (49)	24 (71)	26 (76)
Unable to determine‡	19 (22)	16 (18)	6 (18)	6 (18)
Duration of response, months				
Median (95% CI)	11.4 (3 to 17)	7.4 (2 to 14)	8 (NE to NE)	8 (NE to NE)
Minimum, maximum	2§, 17	2, 19§	8, 8	8, 8

Abbreviations: auto-HCT, autologous hematopoietic cell transplantation; INV, investigator; IRC, independent radiology review committee; NE, not estimable.

*Complete remission or partial remission.

†Nonresponders included patients who had stable disease, progressive disease, or whose status was undetermined.

‡Patients who died or who went off study before the first radiographic assessment.
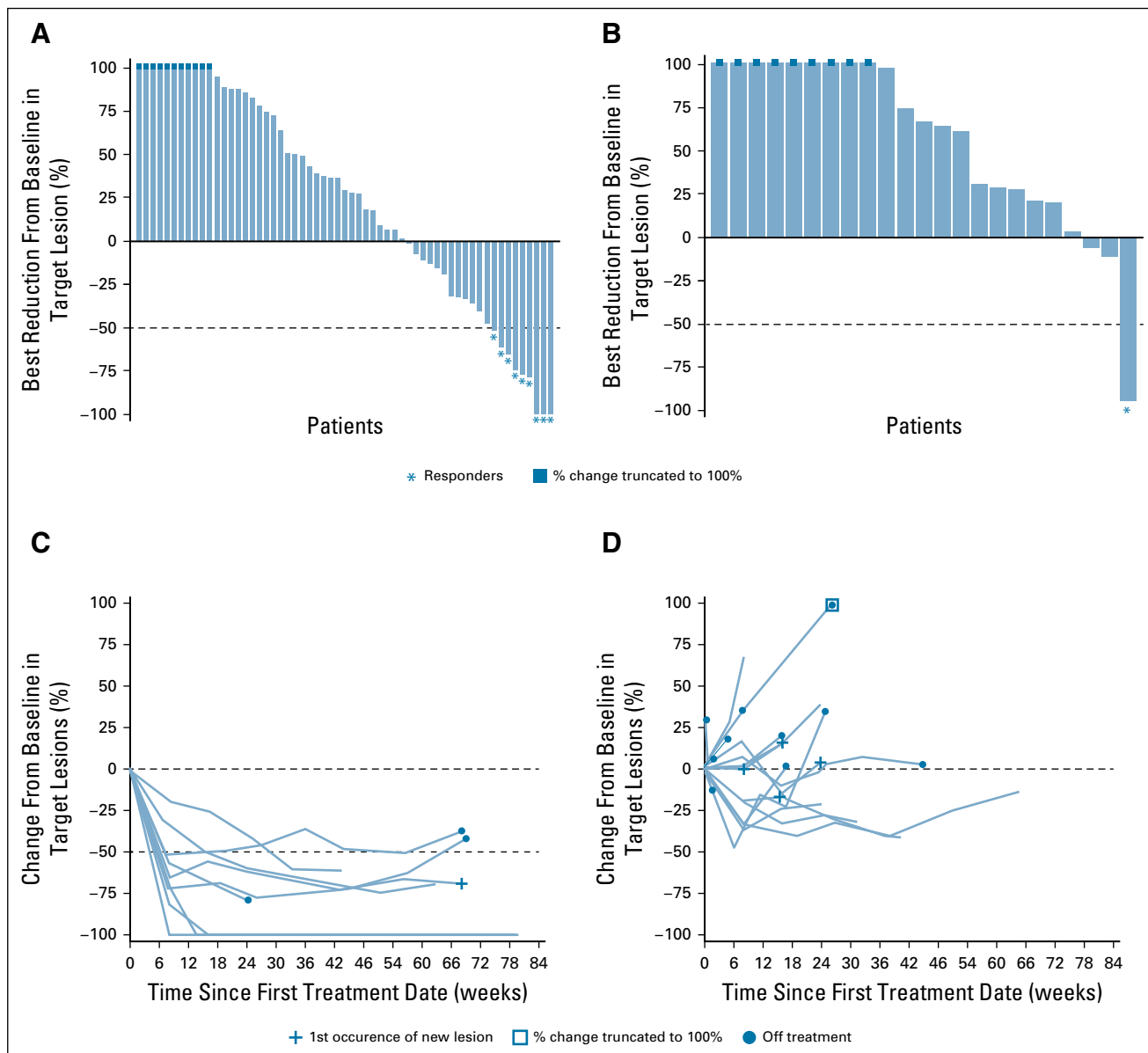
§Censored value with ongoing response.

FIG 1. Change in target lesion burden per independent radiology review committee by best overall response in all response-evaluable patients. Best reduction in target lesion in patients (A) who experienced failure with autologous hematopoietic cell transplantation (auto-HCT) and (B) who were ineligible for auto-HCT. Tumor burden change over time in patients (n = 9) of the auto-HCT–failed cohort who had (C) complete remission (CR) or partial remission (PR) and (D) stable disease (SD; n = 15). Horizontal reference line indicates 50% reduction consistent with a response per revised 2007 International Working Group criteria. Response-evaluable patients had assessments at baseline and at one or more postbaseline time point.

transformed lymphoma. Among the seven patients who were still on treatment, two had CR, two PR, and three stable disease. Nineteen patients in the auto-HCT–failed cohort and five patients in the auto-HCT–ineligible cohort were treated with nivolumab beyond disease progression—none achieved response after progression.

Median PFS was 1.9 months in the auto-HCT–failed cohort and 1.4 months in the auto-HCT–ineligible cohort, with a 6-month IRC-assessed PFS rate of 19.1% and 5.2%, respectively (Fig 2A). Median OS was 12.2 months in the auto-HCT–failed cohort and 5.8 months in the auto-HCT–ineligible cohort, and 6-month OS rate was 67% and 47%, respectively (Fig 2B).

Safety

Grade 3 or 4 AEs were reported in 62% of all treated patients, of which 24% were deemed to be treatment related; none had a frequency of more than 5% (Table 3). The most common treatment-related AEs (TRAEs) were nausea (17%), fatigue (17%), and diarrhea (10%) —these were generally not severe (majority grade 1 to 2). Treatment-related SAEs were reported in 14 patients (12%).
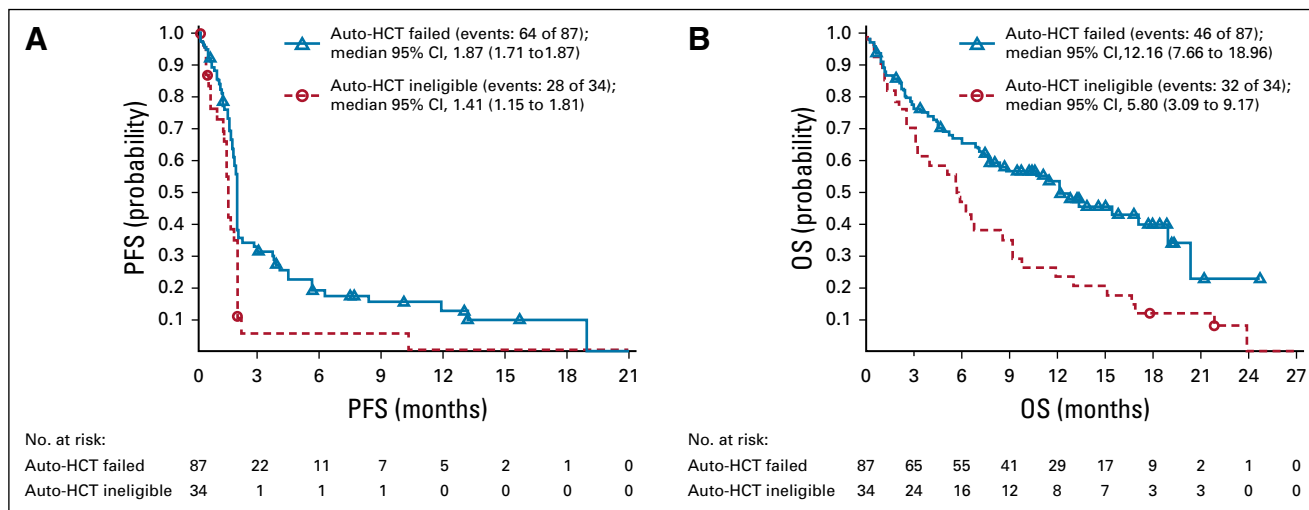
FIG 2. (A) Progression-free survival (PFS) and (B) overall survival (OS) as assessed by independent radiology review committee. Symbols represent censored observations. auto-HCT, autologous hematopoietic cell transplantation.

Four patients (3%) had TRAEs as the primary cause of discontinuation of treatment. TRAEs that contributed to treatment discontinuation included neutropenia, thrombocytopenia, diarrhea, pancreatitis, lipase increase, and dermatitis psoriasiform.

Rates of immune-mediated AEs were low in all treated patients with extended follow-up. The most common grade 3 to 4 immune-mediated AEs were nephritis and renal dysfunction (4%), hepatitis and hepatic dysfunction (3%), diarrhea (3%), and rash (2%).

Overall, 78 patients died. The primary reason for death was disease progression in 39 (45%) and 29 (85%) patients in the auto-HCT–failed and auto-HCT–ineligible cohorts, respectively. No death was attributable to study drug toxicity.

TABLE 3. Drug-Related Adverse Events With an Incidence of ≥ 5%

Adverse Event	All Treated Patients (N = 121)	
	Any Grade	Grade 3 and 4
Any adverse event	118 (98)	75 (62)
Drug-related adverse event	75 (62)	29 (24)
Nausea	21 (17)	1 (< 1)
Fatigue	20 (17)	2 (2)
Diarrhea	12 (10)	1 (< 1)
Neutropenia	9 (7)	5 (4)
Thrombocytopenia	7 (6)	4 (3)
Decreased appetite	7 (6)	1 (< 1)
Lipase increased	6 (5)	4 (3)
Rash	6 (5)	1 (< 1)
Pyrexia	6 (5)	1 (< 1)

NOTE. Data presented as No. (%).

FISH and Immunohistochemistry

In total, 74 of 112 patients had evaluable tumor biopsy specimens for 9p24.1 FISH; 34% of DLBCL specimens were disomic and 45% were polysomic at *PD-L1/PD-L2*. *PD-L1/PD-L2* polysomy in these DLBCLs was low level, largely only three copies in these tumors. Only 16% of DLBCLs exhibited copy gain, which was also low level in the majority of cases (three copies in nine of 13 cases). An additional 3% of tumors had amplification, including *PD-L2*–selective amplification in one patient. Patients were classified by the highest-level 9p24.1 alterations (Figs 3A and 3B), as previously described.[11,23] DLBCLs with 9p24.1 amplification had additional tumor cells with copy gain (0% to 86%) and/or disomy (4% to 8%). Tumors with relative copy gain had additional cells with polysomy (2% to 58%) and/or disomy (2% to 82%), and DLBCLs that were categorized as polysomic had additional disomic tumor cells (14% to 98%; Fig 3B). The percentage of residual disomic cells was lowest in DLBCLs with amplification, intermediate in tumors with copy gain, and highest in tumors with polysomy ($P < .001$; Fig 3C), which is consistent with an ordered spectrum of 9p24.1 genetic alterations.

In this series of DLBCLs with infrequent low-level 9p24.1 alterations, membranous PD-L1 expression was only detected in four (9%) of 46 evaluable cases. One of the three patients who achieved CR had high-level 9p24.1 amplification, whereas the other two had normal 9p24.1 copy number or an unavailable biopsy specimen. Of the seven patients who achieved PR, five had available biopsy specimens and either normal 9p24.1 copy numbers (one patient), low-level polysomy (three patients), or copy gain (one patient). The five evaluable patients with PR had no detectable PD-L1 expression on tumor cells (Appendix Table A1).
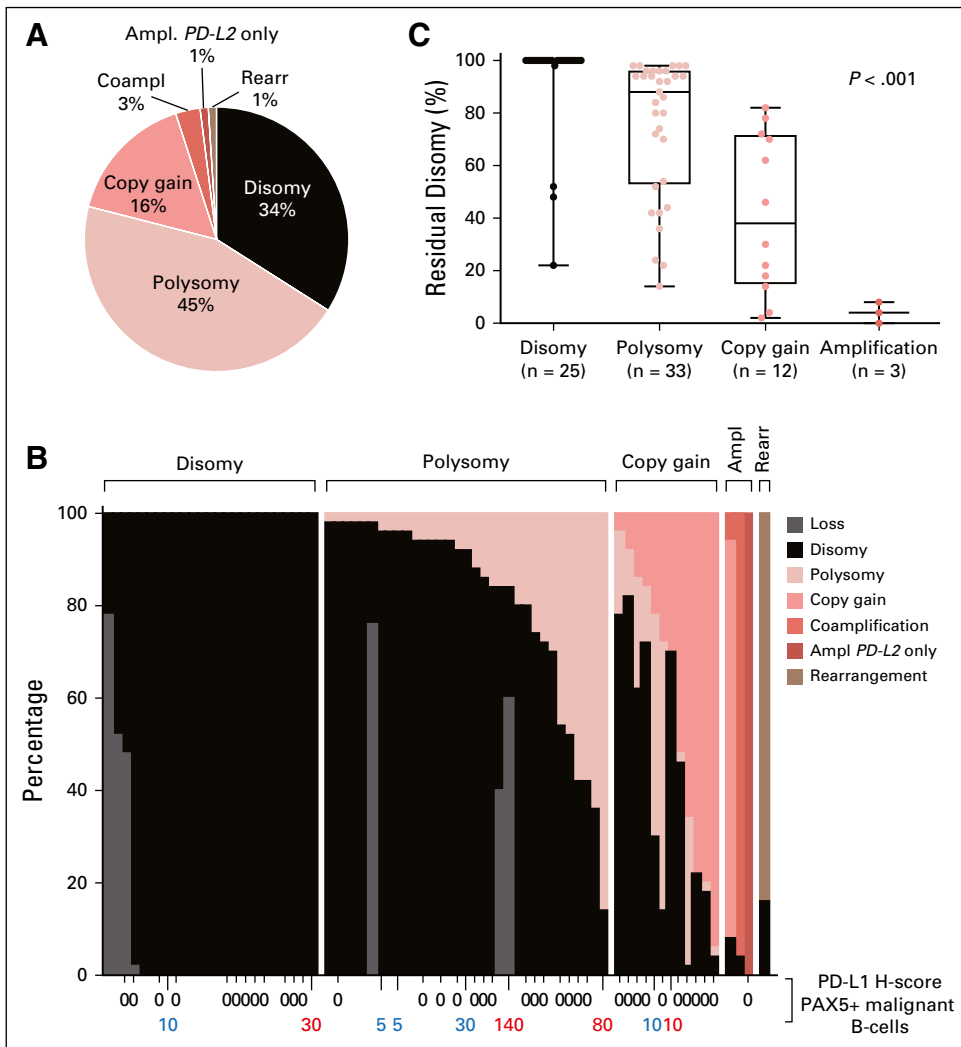
FIG 3. Prevalence and spectrum of 9p24.1 genetic alterations and association of residual disomy with 9p24.1 genetic categories. (A) Prevalence of 9p24.1 genetic alterations in evaluated diffuse large B-cell lymphomas (DLBCLs). (B) Spectrum of 9p24.1 alterations in evaluated DLBCLs. Each patient is classified by the highest observed level of 9p24.1 alteration in tumor cells: polysomy, copy gain, amplification (Ampl), or rearrangement (Rearr). Individual patients are visualized as columns on the x-axis. Percentages of tumor cells with monosomy/relative copy loss (gray), disomy (black), polysomy (light red), copy gain (medium red), amplification (dark red), and rearrangement (brown) are depicted on the y-axis. In cases with evaluable 9p24.1 status and PD-L1 immunohistochemistry (n = 46), programmed death ligand 1 (PD-L1) expression (H-score) on PAX5-positive malignant B cells is indicated below the x-axis (membranous PD-L1 in red, cytoplasmic PD-L1 in blue). (C) Percentage of tumor cells with residual 9p24.1 disomy in DLBCLs classified by 9p24.1 genetic categories (P < .001 from ordinary one-way analysis of variance of unpaired t test data). Coampl, coamplification; PD-L2, programmed death ligand 2.

DISCUSSION

In this phase II study, nivolumab monotherapy was associated with a low incidence of objective response in patients with relapsed/refractory DLBCL, and the study thus did not meet the primary end point. No new safety concerns were identified compared with previous studies that involved patients with solid tumors, HL, and non-HL, acknowledging that most patients received a limited number of doses.[19,21,27]

ORR was lower in the current phase II study than in the phase I study of patients with DLBCL who had received nivolumab monotherapy.[21] This result may be attributed, in part, to differences in study design and patient population. We observed a higher response rate by investigator assessment than by IRC assessment in the phase II study population (Table 2). Centralized IRC assessment on the basis of imaging scans offers standardized and more objective evaluation. In addition, the number of patients with relapsed/refractory DLBCL (n = 11) was low in the phase I study, leading to wide CIs around observed response rates.[21]

Although ORR was low in this study, three patients who achieved CR had DORs of 17 months, 11 or more months, and 14 or more months; none of them had experienced progression at the data cutoff. Two were still alive at 30 and 38 months from first dose with their most recent survival status; however, the number of responders was too low to permit analysis of OS in this group. Whereas PD-L1/PD-L2 amplification in malignant cells was observed for one of these patients, the biologic basis for response in the other two patients is unclear.

Median number of nivolumab doses received (four in the auto-HCT–failed group and three in the auto-HCT–ineligible group) by patients with DLBCL was much lower than those received (16 and 17 doses) in studies that involved patients with relapsed/refractory HL,[19,20] as the majority of the patients with DLBCL stopped treatment because of disease progression. Although it is possible that the rapid progression of DLBCL did not allow enough time for nivolumab to show an effect, we did not observe clear

improvement of responses in the limited number of patients who had been treated beyond disease progression.

The study protocol included a prespecified stopping rule for futility but, on the basis of the safety profile of nivolumab and promising efficacy results observed in the phase I study, allowed enrollment to continue while the interim analysis result was pending. As a result of rapid accrual and delays related to IRC, the planned enrollment was reached before the futility analysis was concluded. Nevertheless, the majority of patients received only a short duration of nivolumab treatment and were allowed to switch to other cancer therapies upon discontinuation of the study treatment; there was no new safety signal observed. In the future, we shall ensure that study procedures are closely monitored to avoid potential delays in the timely completion of the interim analysis.

Previously reported data suggest that PD-L1 expression is associated with poor prognosis in patients with DLBCL[8]; however, the prevalence of PD-L1 expression on DLBCL tumor cells seemed to be low (11%; with a 30% threshold) in that series,[8] as well as in a EBV-negative DLBCL series (11%; with a 5% threshold).[10] The 9p24.1 FISH analyses on a large number of DLBCL trial cases in this study indicate that the incidence and magnitude of 9p24.1 alterations are significantly lower in DLBCL than in cHL, as is the level of PD-L1 expression[20,23] (H-score; Appendix Fig A2, online only). These observations are consistent with the observed differences in the efficacy of PD-1 blockade in DLBCL and cHL and provide a possible explanation for why only a small proportion of patients with relapsed/refractory DLBCL benefit from nivolumab monotherapy. As a result of the low number of responders, there were insufficient data to accurately assess double-hit or c-Myc association with responses in this study.

Whereas the single-agent activity of PD-1 blockade was low in this unselected series of patients with DLBCL, it may be useful to evaluate the approach in select LBCL subtypes with increased PD-L1 expression, such as primary mediastinal B-cell lymphoma, EBV-positive LBCL, and T-cell/histiocyte-rich LBCL.[10] Recent reports suggest that combination treatment strategies, such as chimeric antigen receptor T cells and nivolumab[28] and PD-1 inhibition with concomitant radiotherapy,[29] might also be considered.

In conclusion, the results of this phase II study indicate that nivolumab monotherapy has a low response rate but may provide benefit in a small number of patients with DLBCL who have experienced failure with auto-HCT or who are ineligible for auto-HCT and is associated with a favorable safety profile. Additional research on the influence of biologic factors, including the tumor microenvironment, is warranted to characterize whether a subset of patients with DLBCL may be more likely to respond to nivolumab-based treatment, if combination therapy can improve the therapeutic activity of PD-1 blockade, or if earlier use of these agents before intensive immunoablative chemotherapy may be more effective.

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REFERENCES

1. International Agency for Research on Cancer: World Cancer Report 2014: Diffuse Large B-cell Lymphoma. Geneva, Switzerland, WHO Press, 2014

2. Friedberg JW: Relapsed/refractory diffuse large B-cell lymphoma. Hematology (Am Soc Hematol Educ Program) 2011:498-505, 2011

3. Crump M, Neelapu SS, Farooq U, et al: Outcomes in refractory diffuse large B-cell lymphoma: Results from the international SCHOLAR-1 study. Blood 130:1800-1808, 2017

4. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Non-Hodgkin's lymphomas, version 4.2014. https://www.nccn.org/about/nhl.pdf

5. Nagle SJ, Woo K, Schuster SJ, et al: Outcomes of patients with relapsed/refractory diffuse large B-cell lymphoma with progression of lymphoma after autologous stem cell transplantation in the rituximab era. Am J Hematol 88:890-894, 2013

6. Neelapu SS, Locke FL, Bartlett NL, et al: Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 377:2531-2544, 2017

7. Freeman GJ, Long AJ, Iwai Y, et al: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192:1027-1034, 2000

8. Kiyasu J, Miyoshi H, Hirata A, et al: Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. Blood 126:2193-2201, 2015

9. Green MR, Monti S, Rodig SJ, et al: Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood 116:3268-3277, 2010

10. Chen BJ, Chapuy B, Ouyang J, et al: PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res 19:3462-3473, 2013

11. Roemer MG, Advani RH, Ligon AH, et al: PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. J Clin Oncol 34:2690-2697, 2016

12. Lenz G, Wright GW, Emre NC, et al: Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA 105:13520-13525, 2008

13. Andorsky DJ, Yamada RE, Said J, et al: Programmed death ligand 1 is expressed by non-Hodgkin lymphomas and inhibits the activity of tumor-associated T cells. Clin Cancer Res 17:4232-4244, 2011

14. Bristol-Myers Squibb: Nivolumab (Opdivo) prescribing information. https://packageinserts.bms.com/pi/pi_opdivo.pdf

15. Weber JS, D'Angelo SP, Minor D, et al: Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. Lancet Oncol 16:375-384, 2015

16. Borghaei H, Paz-Ares L, Horn L, et al: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373:1627-1639, 2015

17. Motzer RJ, Escudier B, McDermott DF, et al: Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 373:1803-1813, 2015

18. Sharma P, Callahan MK, Bono P, et al: Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): A multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. Lancet Oncol 17:1590-1598, 2016

19. Ansell SM, Lesokhin AM, Borrello I, et al: PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 372:311-319, 2015

20. Younes A, Santoro A, Shipp M, et al: Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: A multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol 17:1283-1294, 2016

21. Lesokhin AM, Ansell SM, Armand P, et al: Nivolumab in patients with relapsed or refractory hematologic malignancy: Preliminary results of a phase Ib study. J Clin Oncol 34:2698-2704, 2016

22. Cheson BD, Pfistner B, Juweid ME, et al: Revised response criteria for malignant lymphoma. J Clin Oncol 25:579-586, 2007

23. Roemer MGM, Redd RA, Cader FZ, et al: Major histocompatibility complex class II and programmed death ligand 1 expression predict outcome after programmed death 1 blockade in classic Hodgkin lymphoma. J Clin Oncol 36:942-950, 2018

24. Mahoney KM, Sun H, Liao X, et al: PD-L1 antibodies to its cytoplasmic domain most clearly delineate cell membranes in immunohistochemical staining of tumor cells. Cancer Immunol Res 3:1308-1315, 2015

25. Xu-Monette ZY, Thompson D, LaFleur B, et al: Diffuse large B-cell lymphoma cell of origin determination from formalin-fixed paraffin-embedded tissues. Blood 126:5052, 2015 (suppl)

26. US National Cancer Institute: Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Bethesda, MD, National Institutes of Health, 2009

27. Topalian SL, Hodi FS, Brahmer JR, et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366:2443-2454, 2012

28. Hill BT, Roberts ZJ, Rossi JM, et al: Marked re-expansion of chimeric antigen receptor (CAR) T cells and tumor regression following nivolumab treatment in a patient treated with axicabtagene ciloleucel (axi-cel; KTE-C19) for refractory diffuse large B cell lymphoma (DLBCL). Blood 130:2825, 2017 (suppl)

29. Wight JC, Hawkes EA, Berlangieri SU, et al: An abscopal effect may augment PD-1 inhibition in refractory classical Hodgkin lymphoma. Leuk Lymphoma 10.1080/10428194.2018.1452217 [epub ahead of print on March 23, 2018]

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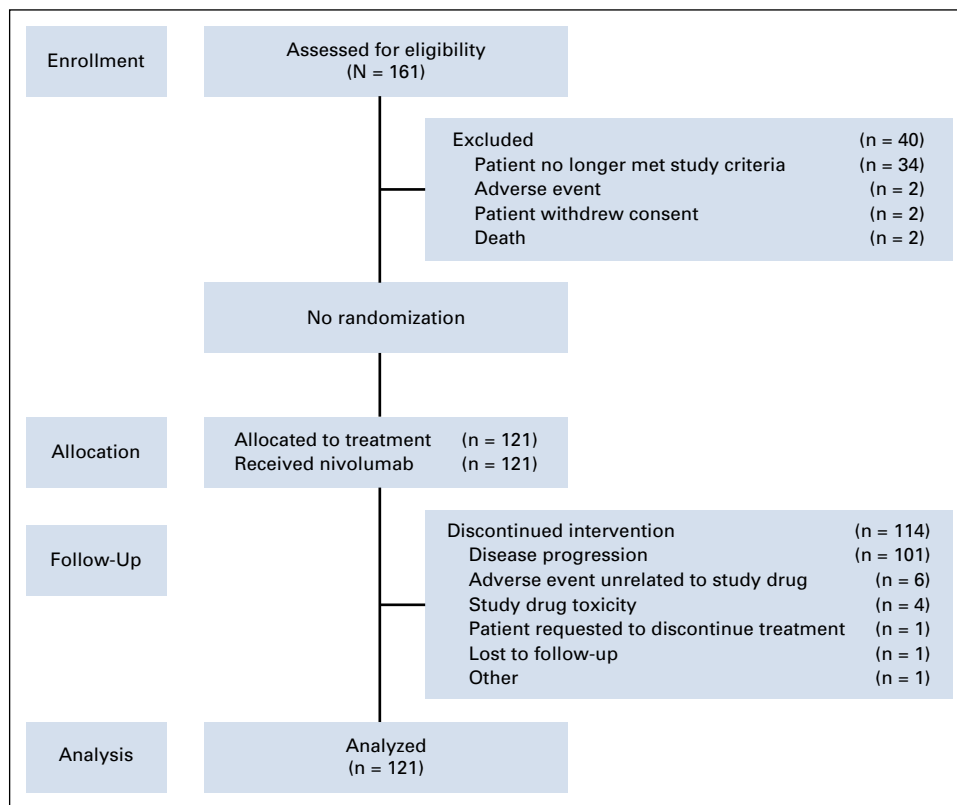No other potential conflicts of interest were reported

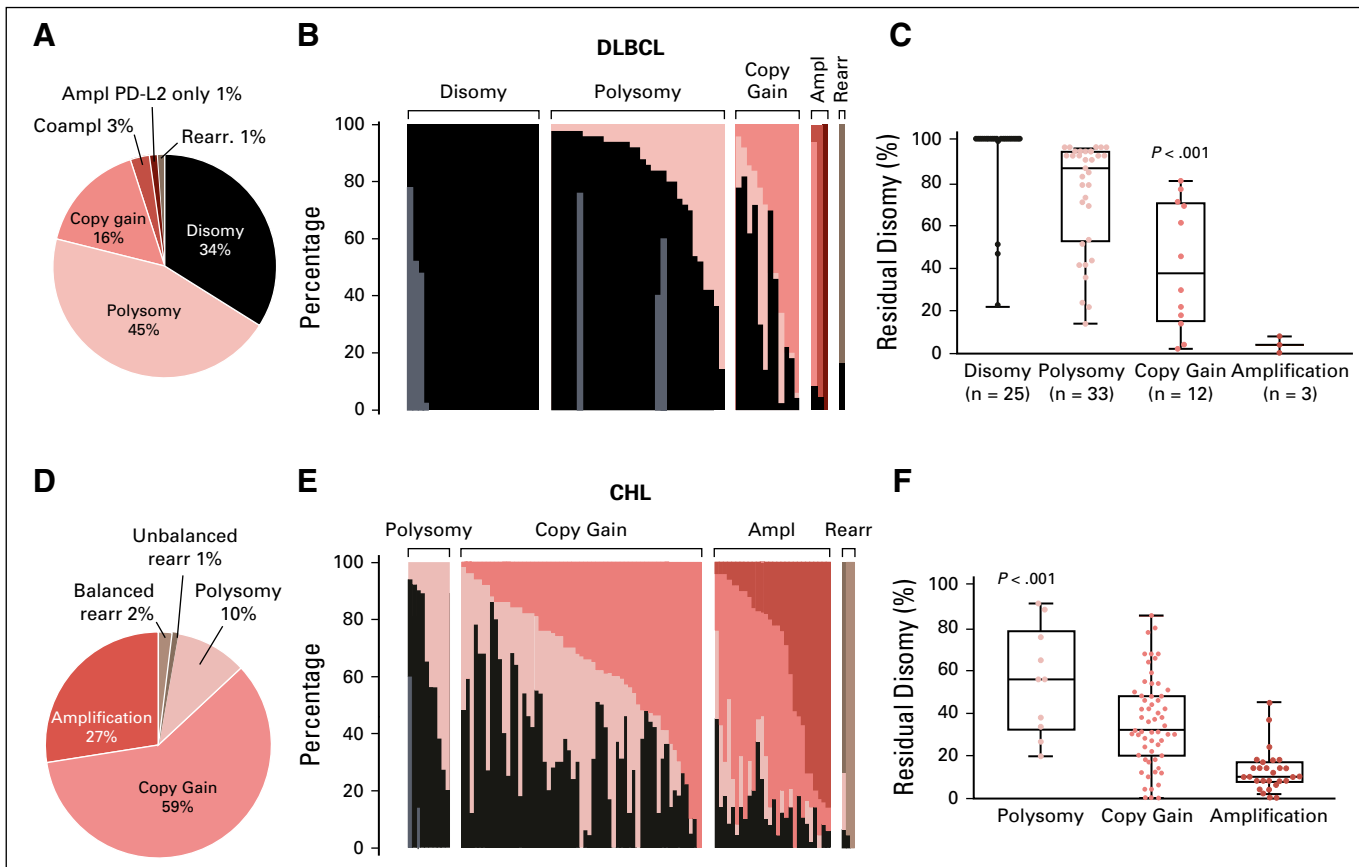**APPENDIX**

FIG A1. Flow diagram.

FIG A2. Comparison of the incidence and magnitude of 9p24.1 alterations in diffuse large B-cell lymphoma (DLBCL; this study) and classic Hodgkin lymphoma (cHL).[23] (A) Prevalence and (B) spectrum of 9p24.1 alterations in DLBCL. (C) Percentage of tumor cells with residual 9p24.1 disomy in DLBCLs classified by 9p24.1 genetic categories. (D) Prevalence and (E) spectrum of 9p24.1 alterations in cHL.[23] (F) Percentage of tumor cells with residual 9p24.1 disomy in cHLs classified by 9p24.1 genetic categories.[23] As noted, DLBCLs have a much lower incidence of 9p24.1 copy gain and amplification than cHLs. In addition, DLBCLs with low-level 9p24.1 alterations have a higher percentage of residual disomy than cHLs. Ampl, amplification; Coampl, coamplification; PD-L2, programmed death ligand 2; Rearr, rearrangement. [Reprinted with permission. © 2018 American Society of Clinical Oncology.]

TABLE A1. Biomarker Data for Patients With Clinical Response (CR and PR)

Cohort and BOR	PFS, Months	DOR, Months	9p24.1 Alteration			% PD-L1 Positive		COO Status	EBV Status
			Alteration	% Cells	Copies, No.	Malignant Cells	Nonmalignant Cells		
HCT failed									
CR	19	17.1	NA			NA	NA	GCB	NA
CR	15.7*	13.8*	Coamplification	96	6 - >15	NA	NA	Non GCB	Negative
CR	13.1*	11.3*	Disomy			0	10 (cytoplasmic)	NA	Negative
PR	13.1	11.4	Polysomy	58	3-4	0	40 (membrane)	GCB	Negative
PR	8.3	6.6	Polysomy	6	3	NA	NA	Non GCB	Negative
PR	11.8	6.3	Polysomy	16	3	NA	NA	GCB	NA
PR	5.6	3.7	Cogain	28	3-4	0	40 (cytoplasmic/ membrane)	Non GCB	Negative
PR	4.4	2.5	NA/disomy†			0	10 (cytoplasmic)/ 30 (membrane)†	GCB	Negative
PR	10*	2.4*	NA			0	50 (cytoplasmic/ membrane)/ 50 (membrane)†	GCB	Negative
HCT ineligible									
PR	10.2	8.3	NA			0	70 (cytoplasmic)	Non GCB	Negative

Abbreviations: BOR, best of response; COO, cell of origin; CR, complete remission; DOR, duration of response; EBV, Epstein-Barr virus; GCB, germinal center B cell; HCT, hematopoietic cell transplantation; NA, not available; PD-L1, programmed death ligand 1; PFS, progression-free survival; PR, partial remission.

*Ongoing response.

†Two samples from the same patient were analyzed.