Chapter 6


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In preparation
Abstract

**Background.** Malignant Hodgkin Reed-Sternberg cells of classical Hodgkin lymphoma (cHL) harbor frequent 9p24.1/PD-L1/PD-L2 copy number alterations (CNAs), leading to overexpression of the PD-1 ligands. This results in inhibition of T cell activation and anti-tumor immune responses. The high response rates of cHL to PD-1 blockade prompted us to determine the prevalence and type of 9p24.1 genetic alterations and PD-L1 expression in relapsed/refractory (R/R) cHL patients treated with nivolumab (anti-PD-1) and assess the association of these parameters with clinical outcome.

**Methods.** In this phase 2 study, patients who had R/R cHL following autologous stem cell transplantation (ASCT) and subsequent brentuximab vedotin (BV) and patients with R/R cHL following ASCT and BV pre- or post-ASCT were enrolled. Genetic alterations on chromosome 9p24.1 were analyzed by fluorescent in situ hybridization assay with probes covering CD274/PD-L1 and PDCD1LG2/PD-L2 and a control centromeric probe. PD-L1 expression was analyzed by immunohistochemistry. We determined the association between 9p24.1 genetic alterations and PD-L1 expression and outcome parameters including best overall response (BOR) and progression-free survival (PFS).

**Results.** All cHL patients in this study had 9p24.1 CNAs, ranging from polysomy (10%) to copy gain (59%) to amplification (28%) and associated increased PD-L1 expression. Patients with high-level 9p24.1 genetic alterations had significantly better overall responses to nivolumab and a longer PFS. Similarly, patients with high PD-L1 expression had a higher complete response rate and longer PFS, while patients with low PD-L1 expression were more likely to have progressive disease and had shorter PFS.

**Conclusion.** Although further research is needed, these data indicate that 9p24.1 genetic alterations and PD-L1 expression may be prognostic biomarkers for response to PD-1 blockade in cHL.
Introduction

Classical Hodgkin lymphomas (cHLs) include infrequent malignant Hodgkin Reed-Sternberg (HRS) cells within an extensive but ineffective inflammatory/immune cell infiltrate.\textsuperscript{1-3} HRS cells exhibit frequent copy number alterations (CNAs) of 9p24.1/CD274(PD-L1)/PDCD1LG2(PD-L2), ranging from low-level polysomy to relative copy gain and high-level amplification and increased expression of programmed death receptor-1 (PD-1) ligands.\textsuperscript{1,4} PD-1 ligands engage the PD-1 receptor on T cells, inhibiting T cell activation and anti-tumor immune responses.\textsuperscript{5} CHL patients with the highest-level 9p24.1 alterations, PD-L1/PD-L2 amplification, have inferior progression-free survival (PFS) after standard primary chemotherapy.\textsuperscript{4}

Currently, only half of the patients with relapsed cHL are cured with salvage therapies. For relapsed/refractory (R/R) patients, treatment options include salvage chemotherapy, autologous stem cell transplantation (ASCT) and treatment with brentuximab vedotin (BV).\textsuperscript{6,7} For patients who progress following ASCT and BV, treatment options are limited. The overexpression of PD-1 ligands in cHL suggested that this disease has genetically determined vulnerability to PD-1 blockade. Indeed, in phase I/II trials of PD-1 blockade with nivolumab or pembrolizumab in relapsed/refractory (R/R) cHL, response rates of 65-87% were seen.\textsuperscript{8-11} Given the demonstrated responsiveness of cHL to PD-1 blockade, we examined the prevalence and type of 9p24.1 genetic alterations, PD-L1 expression, and association of these alterations with clinical outcome in R/R cHL patients receiving nivolumab (anti-PD-1) following ASCT and BV.

Methods

Clinical Data
CheckMate205 is a multicenter, multicohort, phase 2 trial of nivolumab in R/R cHL (Figure 1). This analysis focused on 2 cohorts: patients with recurrent cHL following autologous stem cell transplantation (ASCT) and subsequent brentuximab vedotin (BV) (cohort B), and patients with R/R cHL following ASCT and BV given pre- or post-ASCT (cohort C). Patients received nivolumab 3 mg/kg every 2 weeks. Best overall response (BOR) and PFS were assessed by an independent radiological review committee (IRRC).

Fluorescent in situ hybridization (FISH)
In patients with available tumor biopsies, 9p24.1 genetic alterations were evaluated via fluorescent in situ hybridization (FISH) assay; probes encompassed CD274 (PD-L1, red) or PDCD1LG2 (PD-L2, green) and included a centromeric control probe.
• Phase 2, multicenter, multicohort, single-arm study conducted in Europe and North America\(^a\)
• Inclusion criteria: adults (aged \(\geq 18\) years) with ECOG PS < 2

### Primary:
- ORR assessed by IRRC

### Secondary and exploratory:
- IRRC-assessed DOR and PFS
- Investigator-assessed ORR and DOR
- OS
- Safety
- QoL
- Biomarkers

**Study endpoints**

<table>
<thead>
<tr>
<th>Cohort A</th>
<th>Cohort B</th>
<th>Cohort C</th>
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<tbody>
<tr>
<td>BV naive</td>
<td>BV after ASCT</td>
<td>BV before and/or after ASCT</td>
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<tr>
<td>Continue treatment until PD or unacceptable toxicity</td>
<td>Continue treatment until PD or unacceptable toxicity</td>
<td>Stop treatment if persistent CR for 1 year, if relapse, reinitiate nivo</td>
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\(^a\)The study also included a cohort D in newly diagnosed patients that is beyond the scope of this analysis

ASCT = autologous stem cell transplantation; BV = brentuximab vedotin; cHL = classical Hodgkin lymphoma; CR = complete remission; DOR = duration of response; ECOG PS = Eastern Cooperative Oncology Group Performance Status; IRRC = independent radiological review committee; nivo = nivolumab; ORR = objective response rate; OS = overall survival; PD = progressive disease; PFS = progression-free survival; QoL = quality of life; Q2W = every 2 weeks

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**Immunohistochemistry**

Dual immunohistochemical staining of PD-L1 (clone 405.9A1112) and PAX5 (24/Pax-5; BD Biosciences, San Jose, CA) was performed to delineate PD-L1 expression in PAX5\(^{\text{dim}+}\) HRS cells and PAX5\(^-\) cells in the tumor microenvironment, as previously described.\(^4\) A modified PD-L1 H-score (range 0–300) was calculated by multiplying the percentage of PAX5\(^{\text{dim}+}\) (malignant) cells with positive staining (0–100%) and the average intensity of positive staining (1–3+; \(\geq 50\) RS cells counted).
Statistical analysis
IRRC assessment of BOR (best change from baseline in target lesions) was obtained in all response-evaluable patients. BOR was defined as complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD) of target lesions assessed at baseline, and at least one on-study timepoint, with all baseline target lesions assessed. Post-hoc analyses of the association between BOR and 9p24.1 genetic alterations and PD-L1 H-scores were done with the Kruskal-Wallis rank-sum tests for continuous data comparing two or more groups. The modified H-score for PD-L1 expression was divided into four quartiles. IRRC-assessed PFS was defined from the date of first treatment until the date of progression or death. Patients who underwent subsequent anti-cancer treatment were censored at the date of last contact. Time-to-event analyses were performed using the Kaplan-Meier method, and errors were calculated by Greenwood formula. Differences in survival curves were assessed with log-rank tests. All analyses were done with R version 3.2.2. All p-values are nominal.

Results
Patient characteristics
The baseline characteristics of the patients enrolled in cohorts B and C of CheckMate 205 are shown in Table 1. The median ages in cohort B and C are 37 and 32 years, respectively; more than half of the patients in both cohorts are male. Patients received a median of 4 previous lines of therapy (range 3-15 and 2-9 in cohorts B and C, respectively). Seventy-four percent of patients in cohort B and 69 percent of patients in cohort C received prior radiation therapy and all patients received BV. In cohort B, all patients received BV after auto-ASCT. In cohort C, 57 percent of patients received BV after auto-ASCT, 33 percent received BV before auto-ASCT and for 10 percent of the patients the order is unknown.

9p24.1 genetic alterations and PD-L1 expression
In cohorts B and C, 100 patients had evaluable tumor biopsy specimens; all 100 had detectable 9p24.1 alterations: polysomy in 10/100 (10%), copy gain in 59/100 (59%), amplification in 28/100 (28%), and presumptive rearrangement (split-apart FISH signal) in 3/100 (3%) (Figure 2A and 2B). Lower-level 9p24.1 genetic alterations were seen in additional HRS cells individual cHLs (Figure 2B), as previously reported4. Tumors identified as having amplification had additional HRS cells with copy gain (6-80%), polysomy (4-44%) and disomy (2-45%); those classified as having copy gain had additional cells with polysomy (2-61%) and disomy (4-86%) and those categorized as polysomic for 9p24.1 had additional residual disomic HRS cells (20-92%). The percentage of residual disomic cells was lowest in tumors
with amplification, intermediate in tumors with copy gain and lowest in patients with polysomy \((P < .001, \text{Figure 2C})\), consistent with the ordered spectrum of 9p24.1 genetic alterations. Of the tumors with available 9p24.1 genetic data, 98/100 could be analyzed for PD-L1 protein expression. There was a significant association between PD-L1 protein expression \((\text{H-score})\) and the level of 9p24.1 alterations in HRS cells \((P = .001, \text{Figure 2D})\).

**BOR and PFS according to 9p24.1 alterations and PD-L1 H-scores**

We next evaluated the potential associations between BOR, PFS and defined 9p24.1 alterations and PD-L1 H-scores. The level of 9p24.1 CNAs was significantly associated with BOR \((P = .012)\); no patients with PD had 9p24.1 amplification and no patients with CR had polysomy (Figure 3A). We then assessed PFS for cHL patients by 9p24.1 genetic alteration and identified significant differences in outcome \((P = .039, \text{Figure 3B})\). Patients with 9p24.1 amplification had the most favorable PFS with nivolumab, while patients with polysomy had a shorter PFS. Similarly, there was a significant association between PD-L1 H-scores, divided in quartiles, in HRS cells and BOR \((P = 0.007)\); all patients with PD had PD-L1 H-scores in quartiles 1/2, whereas most patients with CR had PD-L1 H-scores in quartile 4. HRS cell PD-L1 H-score \((\text{by quartiles})\) was also significantly associated with PFS \((P = 0.017)\). Patients with PD-L1 H-scores in quartiles 1 and 2 had shorter PFS than those with scores in quartiles 3 and 4 (Figure 3D).
Discussion

In this study, we found that all evaluable patients had genetic alterations of 9p24.1/PD-L1/PD-L2 and copy number-dependent increased expression of PD-L1 in HRS cells. Although high-level alterations of 9p24.1 and increased PD-L1 expression were previously linked with inferior response to standard induction therapy⁴, we now associate these parameters with more favorable outcomes to targeted PD-1 blockade. These analyses also highlight the importance of quantifying and delineating PD-L1 expression in HRS cells and non-malignant cells in the tumor microenvironment in cHL biopsy specimens. By using a double staining of PD-L1 with PAX5, we were able to specifically determine the expression of PD-L1 on HRS
cells. The significance of expression of PD-L1 on non-malignant cells in the tumor microenvironment remains to be determined.

While further research is needed to guide possible use in clinical practice, these data advance understanding of 9p24.1/PD-L1/PD-L2 genetic alterations and PD-L1 expression as prognostic biomarkers for PD-1 blockade in cHL. These findings may also be applicable to other lymphoma subtypes. Alterations of chromosome 9p24.1 and increased expression of the PD-1 ligands have been described in different large B-cell lymphoma subtypes, including primary testicular lymphoma (PTL) and primary central nervous system lymphoma (PCNSL)\(^{13}\), primary mediastinal large B-cell lymphoma (PMBL)\(^{14-17}\) and T-cell histioocyte/rich large B-cell lymphoma\(^{14}\).
PD-1 blockade is currently being evaluated in each of these lymphoid malignancies. Single agent studies in patients with relapsed/refractory PCNSL and PTL (www.clinicaltrials.gov, NCT02857426) and PMBL (www.clinicaltrials.gov, NCT02576990) are currently underway.

The exact mechanism of action of PD-1 blockade in cHL has not been fully characterized. In solid tumors, CD8+ T-cell responses have been implicated in the response to PD-1 blockade. CD8+ T cells recognize tumor antigens presented by MHC class I on the surface of the tumor cell. While there are clearly clinical responses to PD-1 blockade in the majority of the cHL patients, frequent absent/decreased expression of β2M/MHC class I has been described in cHL, resulting in impaired antigen presentation to CD8+ T cells. Furthermore, antigen presentation via MHC class II to CD4+ effector cells has been implicated in responses to PD-1 blockade in solid tumors. However, in cHL, absent/decreased MHC class II expression has also been described. The consequences of decreased MHC class I and class II-mediated antigen presentation for responses to PD-1 blockade in cHL are currently under investigation.

References