Major Histocompatibility Complex Class II and Programmed Death Ligand 1 Expression Predict Outcome After Programmed Death 1 Blockade in Classic Hodgkin Lymphoma

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ABSTRACT

Purpose
Hodgkin Reed-Sternberg (HRS) cells evade antitumor immunity by multiple means, including gains of 9p24.1/CD274 (PD-L1)/PDCD1LG2 (PD-L2) and perturbed antigen presentation. Programmed death 1 (PD-1) receptor blockade is active in classic Hodgkin lymphoma (cHL) despite reported deficiencies of major histocompatibility complex (MHC) class I expression on HRS cells. Herein, we assess bases of sensitivity to PD-1 blockade in patients with relapsed/refractory cHL who were treated with nivolumab (anti–PD-1) in the CheckMate 205 trial.

Methods
HRS cells from archival tumor biopsies were evaluated for 9p24.1 alterations by fluorescence in situ hybridization and for expression of PD ligand 1 (PD-L1) and the antigen presentation pathway components—β2-microglobulin, MHC class I, and MHC class II—by immunohistochemistry. These parameters were correlated with clinical responses and progression-free survival (PFS) after PD-1 blockade.

Results
Patients with higher-level 9p24.1 copy gain and increased PD-L1 expression on HRS cells had superior PFS. HRS cell expression of β2-microglobulin/MHC class I was not predictive for complete remission or PFS after nivolumab therapy. In contrast, HRS cell expression of MHC class II was predictive for complete remission. In patients with a > 12-month interval between myeloablative autologous stem-cell transplantation and nivolumab therapy, HRS cell expression of MHC class II was associated with prolonged PFS.

Conclusion
Genetically driven PD-L1 expression and MHC class II positivity on HRS cells are potential predictors of favorable outcome after PD-1 blockade. In cHL, clinical responses to nivolumab were not dependent on HRS cell expression of MHC class I.

INTRODUCTION

Classic Hodgkin lymphomas (cHLs) are composed of rare malignant Hodgkin Reed-Sternberg (HRS) cells within an extensive inflammatory and immune cell infiltrate. Despite this T cell–rich infiltrate, HRS cells evade effective antitumor immune responses by multiple mechanisms. HRS cells exhibit frequent copy number alterations of 9p24.1 and the genes encoding the programmed death 1 (PD-1) receptor ligands, PD-L1 and PD-L2 (also called CD274 and PDCD1LG2, respectively), ranging from low-level polysomy to relative copy gain and high-level amplification and copy number–dependent increased expression of PD-1 ligands. PD-1 ligands engage the PD-1 receptor on T cells, inhibiting T-cell activation and antitumor immune responses.

The identified 9p24.1 alterations and PD-1 ligand overexpression in cHL prompted clinical evaluation of PD-1 blockade in patients with...
relapsed/refractory disease. In pilot and registration trials of the PD-1—blocking antibody nivolumab, patients with relapsed/refractory cHL and limited treatment options had response rates of 65% to 87% and prolonged progression-free survival (PFS). Similar results were obtained with a second PD-1—blocking antibody, pembrolizumab. These findings—the highest reported response rates to PD-1 blockade in any tumor type—led to rapid approval by the US Food and Drug Administration and the European Medicines Agency of both PD-1 antibodies for the treatment of relapsed/refractory cHL.

In murine cancer models and human solid tumors, the efficacy of PD-1 blockade has been attributed to activation of CD8+ cytotoxic T cells in the tumor microenvironment. Unlike solid tumors, which are largely MHC class II alterations were evaluated by fl microhistocytology in association with β2-microglobulin (β2M). However, HRS cells exhibit frequent inactivating mutations and copy loss of β2M, leading to decreased or absent cell surface expression of MHC class I. An alternate mechanism of antitumor immunity depends on MHC class II—mediated antigen presentation to CD4+ effector cells. Unlike solid tumors, which are largely MHC class II negative, HRS cells frequently express MHC class II, likely because of their derivation from germinal center B cells. However, a subset of patients with cHL have genetic bases for deficient MHC class II expression and decreased/absent cell surface MHC class II on HRS cells. In recent imaging analyses of the intact cHL tumor microenvironment, we found that PD-L1+ HRS cells were significantly more likely to be in physical contact with PD-1+ CD8+ T cells than PD-L1− CD8+ T cells.

In this study, we assessed the possible predictive value of 9p24.1/PD-L1/PD-L2 genetic alterations and PD-L1 expression for clinical outcome in patients with relapsed/refractory cHL who received nivolumab in the CheckMate 205 trial. We also evaluated HRS cell expression of β2M, MHC class I, and MHC class II and the association of these antigen presentation proteins with outcome after nivolumab therapy.

**METHODS**

**Clinical Data**

CheckMate 205 is a multicenter, multicohort, phase II trial of single-agent nivolumab in patients with cHL. The research protocol was approved by the respective institutional review boards; all participants gave written informed consent. The current biomarker study focused on cohorts B and C: patients with relapsed/refractory cHL who received nivolumab in the CheckMate 205 trial. We also evaluated HRS cell expression of β2M, MHC class I, and MHC class II and the association of these antigen presentation proteins with outcome after nivolumab therapy.

**Fluorescence In Situ Hybridization**

In patients with available archival tumor biopsies, 9p24.1 genetic alterations were evaluated by fluorescence in situ hybridization (FISH) assay; probes encompassed CD274 (PD-L1, red) or PDCD1LG2 (PD-L2, green) and included a centromeric control (aqua). Copy number alterations were defined as previously described on the basis of the target:control signal ratio. Fifty HRS cells per tumor were analyzed. Nuclei with a target:control signal ratio of ≥ 3:1 were defined as coamplified for PD-L1 and PD-L2, and those with a signal ratio of > 1:1 but < 3:1 were classified as having relative copy gain of these loci. Nuclei with a signal ratio of 1:1, but more than two copies per probe, were defined as polysomic for 9p24.1. For each patient, the percentage and magnitude of 9p24.1 amplification, copy gain, polysomy, and normal copy numbers (disomy) were noted. 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.

**Immunohistochemistry**

Dual immunohistochemical staining of PD-L1 (clone 405.9A11) and PD-L2 (24/Pax-5; BD Biosciences, San Jose, CA) was performed to delineate PD-L1 expression in PAX5dim+ malignant HRS cells on archival tumor biopsies, as previously described. A modified PD-L1 H-score (range, 0 to 300) was calculated by multiplying the percentage of PAX5dim+ (malignant) cells with positive PD-L1 staining (0% to 100%) and the average intensity of staining (0 to 3+ on 50 HRS cells). Dual immunohistochemical staining of the antigen presentation components β2M (A0072, 1:6,000; Dako, Taipei, CA), MHC class II (C03/43 M077s, 1:750; Dako) with PAX5 (BD Biosciences) was performed using an automated staining system (Bond III; Leica Biosystems, Vista, CA) according to the manufacturer's protocol.

Scoring criteria for samples in this trial followed the three-tiered scoring system previously established in the analysis of newly diagnosed cHLs treated with standard induction therapy. Briefly, two expert hematopathologists (S.J.R. and G.S.P.) independently reviewed each case. After their initial assessment, the two pathologists reviewed each case together and recorded a single consensus score. Each tumor was classified as positive, decreased, or negative for expression on HRS cells, relative to staining on adjacent nonmalignant inflammatory cells. In each case, areas with adjacent HRS cells were closely analyzed for HRS cell membrane expression of β2M, MHC class I, and MHC class II, a process analogous to that used for the clinical evaluation of CD45 (leucocyte common antigen). For cases categorized as positive, at least 90% of evaluable HRS cells showed positive membrane staining for the biomarker at levels equivalent to, or greater than, that of adjacent nonmalignant inflammatory cells. For cases categorized as negative, at least 90% of evaluable HRS cells showed no detectable membrane staining for the biomarker relative to nonmalignant inflammatory cells. For cases categorized as decreased, positive membrane staining of HRS cells was present and unequivocally reduced relative to surrounding cells, and/or positive staining was observed in < 90% of evaluable HRS cells. Stained slides were scored blinded to the outcome data.

**Statistical Analyses**

IRC assessment of BOR was obtained in all treated patients at the database lock in December 2016. BOR was defined as the best response (complete remission [CR], partial remission [PR], stable disease, progressive disease [PD], or nonevaluable disease) between the first treatment until the date of progression or death. Patients who underwent subsequent anticancer treatment without reported progression were censored at the last tumor assessment before the additional anticancer therapy. Time-to-event analyses were performed using the Kaplan-Meier method, and SEs were calculated by Greenwood's formula. Differences in PFS curves were assessed with log-rank tests. IRC-assessed PFS was defined as time from the date of first treatment until the date of progression or death. Patients who underwent subsequent anticancer treatment without reported progression were censored at the last tumor assessment before the additional anticancer therapy. Time-to-event analyses were performed using the Kaplan-Meier method, and SEs were calculated by Greenwood's formula. Differences in PFS curves were assessed with log-rank tests. IRC-assessed PFS was defined as time from the date of first treatment until the date of progression or death. Patients who underwent subsequent anticancer treatment without reported progression were censored at the last tumor assessment before the additional anticancer therapy.
Patient Characteristics

A total of 180 patients with relapsed/refractory cHL were treated in cohorts B and C of CheckMate 205. All 80 patients in cohort B received single-agent BV after relapsing from ASCT (ASCT→BV). In contrast, cohort C (n = 100) included patients who received BV before ASCT for treatment of relapse (BV→ASCT; n = 33), BV after relapse from ASCT (ASCT→BV, n = 58), or BV before and after relapse from ASCT (BV→ASCT→BV, n = 9; Data Supplement Table A1A and Fig A1).

An identified clinical difference in patients from cohorts B and C was the time (median [range]) between myeloablative ASCT and nivolumab therapy (cohort B, 40 [2 to 228] months; versus cohort C, 21 [3 to 204] months; P < .001; Data Supplement Table A1A). This difference largely reflected the sequence of prior therapies for relapsed disease in the respective cohorts. In the cohort B ASCT→BV and cohort C ASCT→BV and BV→ASCT→BV subsets, time intervals between myeloablative ASCT and nivolumab therapy were 40 months (2 to 228 months), 32 months (3 to 204 months), and 23 months (7 to 40 months), respectively. In the cohort C BV→ASCT subset, the time interval between ASCT and treatment was only 10 months (3 to 42 months) months (P < .001; Data Supplement Table A1A).

We postulated that patients with a shorter interval between myeloablative ASCT and nivolumab therapy were still actively reconstituting their immune repertoire and tumor microenvironment at study entry.25-27 For this reason, we analyzed patients who were treated with nivolumab (ASCT→BV), n = 33), BV after relapse from ASCT (ASCT→BV, n = 58), or BV before and after relapse from ASCT (BV→ASCT→BV, n = 9; Data Supplement Table A1A and Fig A1).

RESULTS

9p24.1 Genetic Alterations and PD-L1 Expression

In total, 99 of 180 patients had evaluable tumor biopsy specimens for 9p24.1 FISH (Data Supplement Table A1B); all 99 had detectable 9p24.1 alterations in HRS cells: polysomy in 10 of 99 (10%), copy gain in 59 of 99 (60%), and amplification in 27 of 99 (27%; Fig 1A). Patients were classified by the highest-level 9p24.1 alterations (Figs 1A and 1B), as previously described.4 Tumors with 9p24.1 amplification had additional HRS cells with copy gain (6% to 84%), polysomy (4% to 44%), and disomy (2% to 45%); those with relative copy gain had additional cells with polysomy (2% to 84%) and disomy (4% to 86%), and those categorized as polysomic had additional residual disomic HRS cells (20% to 92%; Fig 1B). The percentage of residual disomic cells was lowest in tumors with amplification, intermediate in tumors with copy gain, and highest in tumors with polysomy (P < .001; Fig 1C), consistent with an ordered spectrum of 9p24.1 genetic alterations in cHL.

Of patients with available 9p24.1 genetic data, 97 of 99 could be analyzed for PD-L1 protein expression (Data Supplement Table A1B). There was a significant association between PD-L1 protein expression (H-score) and the magnitude of 9p24.1 copy number alterations in HRS cells (P = .001; Fig 1D). Similar results were obtained for patients with intervals of ≤12 or >12 months between ASCT and nivolumab therapy (Data Supplement Fig A3).
In addition to copy number changes, chromosomal rearrangements of 9p24.1 were observed in three of 99 (3%) patients (Figs 1A and 1B). Two (2%) patients had balanced rearrangements by FISH, positive PD-L2 expression, and no PD-L1 immunostaining (Data Supplement Fig A4; patients 1 and 2), indicative of PD-L2 translocations. The third patient (1%) had an unbalanced rearrangement, with extra copies of PD-L1 and selective membranous PD-L1 expression (Data Supplement Fig A4; patient 3).

**BOR and PFS According to 9p24.1 Alterations and PD-L1 H-Scores**

We next evaluated the potential associations between BOR and defined 9p24.1 alterations and PD-L1 H-scores (Figs 2A and 2B). The majority of evaluable patients responded to PD-1 blockade (Figs 2A and 2B). In contrast to patients who obtained CR or PR or had stable disease, those who experienced progression on therapy were more likely to have lower-level 9p24.1 alterations (polysomy; Fig 2A and Data Supplement Fig A5A; PD vs non-PD, \( P = .006 \)) and less PD-L1 expression on HRS cells (Fig 2B and Data Supplement Fig A5B; PD vs non-PD, \( P = .047 \)). Patients whose tumors had lower-level 9p24.1 alterations and less PD-L1 expression on HRS cells also had shorter PFS (Fig 2C, \( P < .001 \), and Fig 2D, \( P = .026 \)).

A patient whose tumor had an unbalanced rearrangement involving PD-L1 obtained a PR and had prolonged PFS (patient 3, 14.8+ months; Data Supplement Fig A4A). The two patients with balanced PD-L2 rearranged tumors had rapidly PD (PFS was 1.4 months in patient 1 and 2.1 months in patient 2; Fig 2C and Data Supplement Fig A4A).
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<td>16 (22%)</td>
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</tr>
<tr>
<td>Positive</td>
<td>5 (7%)</td>
<td>5 (7%)</td>
<td>28 (39%)</td>
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Fig 3. β2-microglobulin (β2M), major histocompatibility complex (MHC) class I and MHC class II expression. (A) β2M, MHC class I, and MHC class II expression in patients in whom all three antigen presentation components were evaluable (n = 72). (B) Representative images from five trial patients. Images include: a low-power (×400) hematoxylin and eosin stain (left panels) with a CD30+ Hodgkin Reed-Sternberg (HRS) cell in the inset (right upper corner); and high-power (×1,000) β2M/PAX5, MHC class I (MHC-I)/PAX5, and MHC class II (MHC-II)/PAX5 double stains (middle and right panels) with representative HRS cells in insets (right upper corner). For cases categorized as positive (Pos), at least 90% of evaluable PAX5dim+HRS cells showed positive membrane staining for β2M, MHC class I, and/or MHC class II at levels equivalent to or greater than that of adjacent nonmalignant inflammatory cells. In negative cases (Neg), at least 90% of evaluable PAX5dim+HRS cells showed no detectable membrane staining for the biomarkers relative to adjacent nonmalignant inflammatory cells. HRS cells from patient 1 were positive (Pos) for β2M and MHC class I and negative (Neg) for MHC class II. This patient had stable disease as best overall response. HRS cells from patients 2, 3, and 4 were negative for β2M and MHC class I and positive for MHC class II. All three patients obtained complete remissions. HRS cells from patient 5 were positive for β2M, MHC class I, and MHC class II. This patient achieved a partial remission. Magnification × 1,000. Scale bar, 50 μm.
In patients with intervals of ≤ 12 or > 12 months between ASCT and nivolumab therapy, the magnitude of 9p24.1 alterations was significantly associated with PFS (Data Supplement Fig A5C-D). The levels of PD-L1 protein expression were significantly associated with PFS in patients with > 12 months between ASCT and nivolumab therapy (Data Supplement Fig A5F) but not in those with a ≤ 12-month interval (Data Supplement Fig A5E). In the latter group of patients, who were still reconstituting their immune repertoire and tumor microenvironment after myeloablative ASCT, PD-L1 expression may have been driven by additional microenvironmental signals that would not be reflected in the analyzed archival tumor biopsies (Data Supplement Fig A5E).

**Antigen Presentation in Patients Treated With Nivolumab**

Given the importance of effective antigen presentation for T-cell responses, we next assessed the patterns of β2M, MHC class I, and MHC class II expression on HRS cells. In total, 72 patients had available specimens for analysis of all three antigen presentation components (Data Supplement Table A1B and Fig 3A; representative images in Fig 3B and Data Supplement Fig A6). HRS cell membrane expression of β2M was negative in 51 of 72 (71%) tumors, decreased in 16 of 72 (22%), and positive in five of 72 (7%; Fig 3A). Similarly, HRS cell membrane expression of MHC class I was negative in 46 of 72 (64%) patients, decreased in 21 of 72 (29%), and positive in five of 72 (7%); Fig 3A). Similarly, HRS cell membrane expression of MHC class II was negative in 46 of 72 (64%) patients, decreased in 21 of 72 (29%), and positive in five of 72 (7%); Fig 3A).

**Fig 4.** Best overall response (BOR) and progression-free survival (PFS) by β2-microglobulin (β2M) and major histocompatibility complex (MHC) class I expression. Total number of evaluable patients for β2M and MHC class I expression was 72. Three patients were not evaluable for BOR in A and B (n = 69). All 72 patients were evaluable for PFS in C and D. BOR according to (A) β2M expression, and (B) MHC class I expression. For each category of expression in A and B, the No. (%) of patients with each BOR are indicated. y-axis denotes the number (and percentages) of total analyzed cases in A and B. (C) PFS by β2M expression (P = .07, log-rank test). (D) PFS by MHC class I expression (P = .38, log-rank test). CR, complete remission; Dec, decreased; Neg, negative; PD, progressive disease; Pos, positive; PR, partial remission; SD, stable disease.

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positive in five of 72 (7%; Fig 3A). In this series, there was a highly significant association between HRS cell expression of β2M and MHC class I, implicating β2M alterations in the perturbed MHC class I presentation (P < .001; Data Supplement Table A2). Overall, 93% (67 of 72) of analyzed cHLs had impaired (negative or decreased) HRS cell surface expression of MHC class I (Fig 3A).

HRS cell membrane expression of MHC class II was negative in 21 of 72 (29%) patients, decreased in 23 of 72 (32%), and positive in 28 of 72 (39%; Fig 3A). There was no association between the expression of MHC class II and β2M or MHC class I on HRS cells (P = .645 and P = .5764, respectively; Data Supplement Table A2).

Antigen Presentation and Clinical Outcome

We next examined the importance of intact MHC class I and II expression on HRS cells for response to PD-1 blockade. First, we assessed the association between BOR, PFS, and β2M and MHC class I expression. Ninety-two percent (11 of 12) of patients who achieved a CR to nivolumab had tumors that were negative for β2M and MHC class I (Figs 4A and 4B). There was no association between β2M or MHC class I expression on HRS cells and PFS (Figs 4C and 4D). Similar results were obtained in patients with intervals of ≤12 and >12 months between ASCT and nivolumab therapy (Data Supplement Fig A7). These data suggest that, in cHL, PD-1 blockade is largely independent of MHC class I–mediated antigen recognition and CD8+ cytotoxic T-cell responses.

In contrast, 92% (11 of 12) of complete responders had tumors with membranous MHC class II expression on HRS cells (67% [eight of 12] positive, 25% [three of 12] decreased; Fig 5A and Data Supplement Fig A8; CR vs no CR, P = .03). MHC class II expression on HRS cells was not predictive for PFS in patients who underwent autologous stem-cell transplantation ≥ 12 months before nivolumab treatment (n = 22; P = .16, log-rank test). MHC class II expression on HRS cells was not predictive for PFS in patients who underwent autologous stem-cell transplantation >12 months before nivolumab treatment (n = 50; P = .014, log-rank test).

![Graphs](image-url)

**Fig 5.** Best overall response (BOR) and progression-free survival (PFS) by major histocompatibility complex (MHC) class II expression. Total number of evaluable patients for MHC class II expression was 72. Three cases were not evaluable for BOR in A (n = 69). All 72 patients were evaluable for PFS in B and C. (A) BOR by MHC class II expression. For each category of expression, the No. (%) of patients with each BOR are indicated. (B) PFS by MHC class II expression in patients who underwent autologous stem-cell transplantation ≤12 months before nivolumab treatment (n = 22; P = .16, log-rank test). (C) PFS by MHC class II expression in patients who underwent autologous stem-cell transplantation >12 months before nivolumab treatment (n = 50; P = .014, log-rank test). CR, complete remission; Dec, decreased; Neg, negative; PD, progressive disease; PD-1, programmed death 1; Pos, positive; PR, partial remission; SD, stable disease.
were treated with nivolumab ≤ 12 months after myeloablative ASCT, likely because of microenvironmental signals that would not be reflected in archival biopsy specimens (Fig 5B). However, in patients who received the PD-1–blocking antibody > 12 months after ASCT, positive MHC class II expression on HRS cells was predictive for prolonged PFS (Fig 5C; P = .014). Taken together, these data highlight a previously unappreciated role of MHC class II–mediated antigen presentation on HRS cells in the clinical response to PD-1 blockade.

**DISCUSSION**

In this study of patients with relapsed/refractory cHL, we assessed the possible predictive value of specific immune evasion mechanisms—9p24.1/PD-L1/PD-L2 alterations, PD-L1 expression, and perturbed antigen presentation—for response to nivolumab therapy as well as PFS after treatment. 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 most patients responded to nivolumab therapy, those with higher-level 9p24.1 alterations and PD-L1 expression on HRS cells had superior PFS. These analyses highlight the importance of quantifying and specifically delineating PD-L1 expression in malignant HRS cells.

Our data suggest that the mechanism of action for PD-1 blockade in cHL may be different from that described in certain solid tumors.10–13 In the current study, 93% of evaluable patients with cHL had absent or decreased expression of β2M and MHC class I. In 92% of complete responders to nivolumab, HRS cells were negative for cell surface β2M and MHC class I; moreover, PFS was unrelated to HRS cell expression of β2M or MHC class I. These findings strongly suggest that β2M deficiency is a major mechanism of MHC class I loss in cHL, and PD-1 blockade is largely independent of MHC class I–mediated antigen recognition and associated CD8+ cytotoxic T-cell responses in cHL.

In marked contrast, 92% of complete responders to nivolumab had membranous MHC class II expression on their HRS cells. The observed variability in MHC class II expression on HRS cells may have genetic bases, such as inactivating rearrangements of the MHC class II transactivator CIITA19 and downregulation of CIITA by Epstein-Barr virus latent membrane protein 2A.20 In patients with fully reconstituted immune systems (> 12 months from myeloablative ASCT), positive MHC class II expression on HRS cells was also predictive for prolonged PFS. Of note, a subset of patients with MHC class II–negative HRS cells also had short-lived responses to nivolumab, suggesting that additional mechanisms remain to be defined.

These initial analyses were limited by the availability of tumor biopsy specimens from patients in the CheckMate 205 trial and should be prospectively validated in additional trials of PD-1 blockade in relapsed/refractory cHL.9 However, our data already prompt speculation regarding an alternative CD4+ T cell–mediated mechanism of response to PD-1 blockade in cHL and suggest associated biomarkers of response and resistance and additional complementary targets, such as the MHC class II-interacting checkpoint protein, LAG3.28

Despite the focus on CD8+ T cell–mediated mechanisms of PD-1 blockade,10–13 recent studies also highlight the role of MHC class II–associated antigen presentation and CD4+–infiltrating T cells in certain solid tumors16 and define tumor neoantigens that are largely recognized by CD4+ T cells.17,18,29 Taken together, these findings support the hypothesis of an alternative MHC class II–dependent, CD4+ T cell–associated mechanism of action of PD-1 blockade in cHL and, possibly, other tumors.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Disclosures provided by the authors are available with this article at jco.org.

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MHC Class II, PD-L1, and Outcome After PD-1 Blockade

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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MHC Class II, PD-L1, and Outcome After PD-1 Blockade

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