

# Classical Hodgkin Lymphoma with Reduced $\beta_2M$ /MHC Class I Expression Is Associated with Inferior Outcome Independent of 9p24.1 Status

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## Abstract

In classical Hodgkin lymphoma (cHL), malignant Hodgkin Reed–Sternberg (HRS) cells evade antitumor immunity by multiple mechanisms, including perturbed antigen presentation and enhanced PD-1 signaling. HRS cell expression of the PD-1 ligands is attributable, in part, to copy number alterations of 9p24.1/*CD274*(*PD-L1*)/*PDCD1LG2*(*PD-L2*). Amplification of *PD-L1*/*PD-L2* is associated with advanced clinical stage and inferior progression-free survival (PFS) following first-line (induction) therapy. The relationships between altered expression of  $\beta_2$ -microglobulin ( $\beta_2M$ ), MHC class I, and MHC class II by HRS cells, *PD-L1*/*PD-L2* amplification, and clinical outcome in cHL are poorly defined. We assessed these variables in diagnostic biopsy specimens from 108 patients with cHL who

received uniform treatment and had long-term follow-up and found decreased/absent expression of  $\beta_2M$ /MHC class I in 79% (85/108) and decreased/absent expression of MHC class II in 67% (72/108) of cases. Patients with decreased/absent  $\beta_2M$ /MHC class I had shorter PFS, independent of *PD-L1*/*PD-L2* amplification and advanced stage. Decreased or absent MHC class II was unrelated to outcome. These results suggest that MHC class I-mediated antigen presentation by HRS cells is an important component of the biological response to standard chemo/radiotherapy. The paucity of  $\beta_2M$ /MHC class I expression on HRS cells also prompts speculation regarding alternative mechanisms of action of PD-1 blockade in cHL. *Cancer Immunol Res*; 4(11): 910–6. ©2016 AACR.

## Introduction

Primary classical Hodgkin lymphomas (cHL) are comprised of a mixed infiltrate of inflammatory/immune cells and small numbers of malignant Hodgkin Reed–Sternberg (HRS) cells (1). HRS cells evade antitumor immunity by multiple mechanisms, including perturbed antigen presentation and augmented PD-1 signaling, that are attributable, in large part, to defined genetic lesions. Recent studies identified HRS cell lines and primary HRS cells with *B2M* mutations that disrupt expression of the  $\beta_2$ -microglobulin ( $\beta_2M$ )/MHC class I dual protein complex at the cell surface (2). Separate studies defined inactivating

alterations of the MHC class II transactivator, *CIITA*, in cHL (3). Finally, in virtually all cases of cHL, HRS cells have copy number alterations of 9p24.1, a region that includes *CD274* (*PD-L1*) and *PDCD1LG2* (*PD-L2*), and contributes to robust expression of the PD-1 ligands (4).

The biological and clinical significance of the varied immune evasion strategies in cHL are still being elucidated. An initial study on clinical samples from patients with cHL suggested that loss of  $\beta_2M$  protein was, paradoxically, associated with improved clinical outcome (2). In contrast, our recent analysis of diagnostic biopsy samples from patients treated with Stanford V, a standard induction regimen comparable with ABVD (Adriamycin, bleomycin, vinblastine, and dacarbazine), revealed highly significant associations between amplification of *PD-L1*/*PD-L2* and advanced stage disease at presentation and inferior progression-free survival (PFS; ref. 4). The genetic basis of PD-1-mediated immune evasion likely explains the efficacy of PD-1 blockade in cHL. In pilot studies and confirmatory phase II trials, patients with relapsed/refractory cHL treated with PD-1-blocking antibodies had response rates of 65% to 87% and long-lasting remissions (5–7).

We sought to clarify the prognostic significance of perturbed MHC class I and MHC class II antigen presentation by HRS cells and explore the relationship between antigen presentation and 9p24.1 genetic alterations in cHL. Herein, we characterize cell-surface  $\beta_2M$ , MHC class I, and MHC class II expression in a series of uniformly treated cHL patients with long-term follow-up and defined 9p24.1/*PD-L1*/*PD-L2* alterations (4).

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**Note:** Supplementary data for this article are available at Cancer Immunology Research Online (<http://cancerimmunolres.aacrjournals.org/>).

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## Materials and Methods

### Patient samples and 9p24.1 genetic analyses

The samples used in this study were from a previously described 108-patient series, with Institutional Review Board approval (4). Formalin-fixed paraffin-embedded (FFPE) tumor samples and select pathologic and clinical data from 108 patients with newly diagnosed cHL, treated with the Stanford V chemotherapy regimen + modified involved field radiation, were obtained from the Stanford University (Stanford, CA; ref. 4). Median patient follow-up was 9 years. In this series, 9p24.1/*PD-L1*/*PD-L2* alterations were characterized by FISH as described previously (4, 8).

### IHC

Immunohistochemical staining for  $\beta_2$ M (Dako, A0072, 1:6,000), MHC class I (Abcam, EMR8-5, 1:6,000), and MHC class II (Dako, CR3/43 M0775, 1:750) was performed using an automated staining system (Bond III, Leica Biosystems) according to the manufacturer's protocol following antigen retrieval (Bond, ER2 solution). Hematoxylin counterstain was subsequently applied.

### Scoring stained tissue sections

Staining on two or more adjacent HRS cells was used to determine membrane expression of the antigen presentation proteins,  $\beta_2$ M, MHC class I, and MHC class II. HRS cells were evaluated for the presence of positive membrane staining of each biomarker. If present, the relative intensity of HRS cell membrane expression, relative to adjacent nonmalignant inflammatory cells, was determined.  $\beta_2$ M, MHC class I, and MHC class II IHC were optimized on a test series of cHLs. In a subset of cases,  $\beta_2$ M, MHC class I, and/or MHC class II expression on the vast majority of HRS membranes was equivalent to or greater than that observed on adjacent, nonmalignant inflammatory cells (Supplementary Fig. S1; case 1 for  $\beta_2$ M and MHC class I, case 2 for MHC class II). In a subset, no  $\beta_2$ M, MHC class I, and/or MHC class II expression was detected on the vast majority of HRS cells or cell membranes, despite appropriate internal controls (Supplementary Fig. S1; case 3 for  $\beta_2$ M and MHC class I, case 1 for MHC class II). An additional subset of cases exhibited heterogeneous HRS cell staining, including HRS cells with unequivocally positive but reduced membrane staining, relative to adjacent nonmalignant cells, and those with a combination of reduced and complete loss of staining in a subset of cells (Supplementary Fig. S1; case 2 for  $\beta_2$ M and MHC class I, case 3 for MHC class II).

We devised a 3-tier scoring system to categorize the predominant patterns of  $\beta_2$ M, MHC class I, and MHC class II expression by HRS cells in each case. 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 less than 90% of evaluable HRS cells.

Stained slides from the clinical series were scored separately for each of the markers by two independent hematopathologists (S.J. Rodig and G.S. Pinkus), blinded to the clinical data. Kappa

statistics were then generated on the two sets of independent scores (Supplementary Table S1). Cases with scores that were found to be discordant between the two independent reviewers were reconciled in a consensus conference. The consensus score was used for the final analysis.

### Statistical analysis

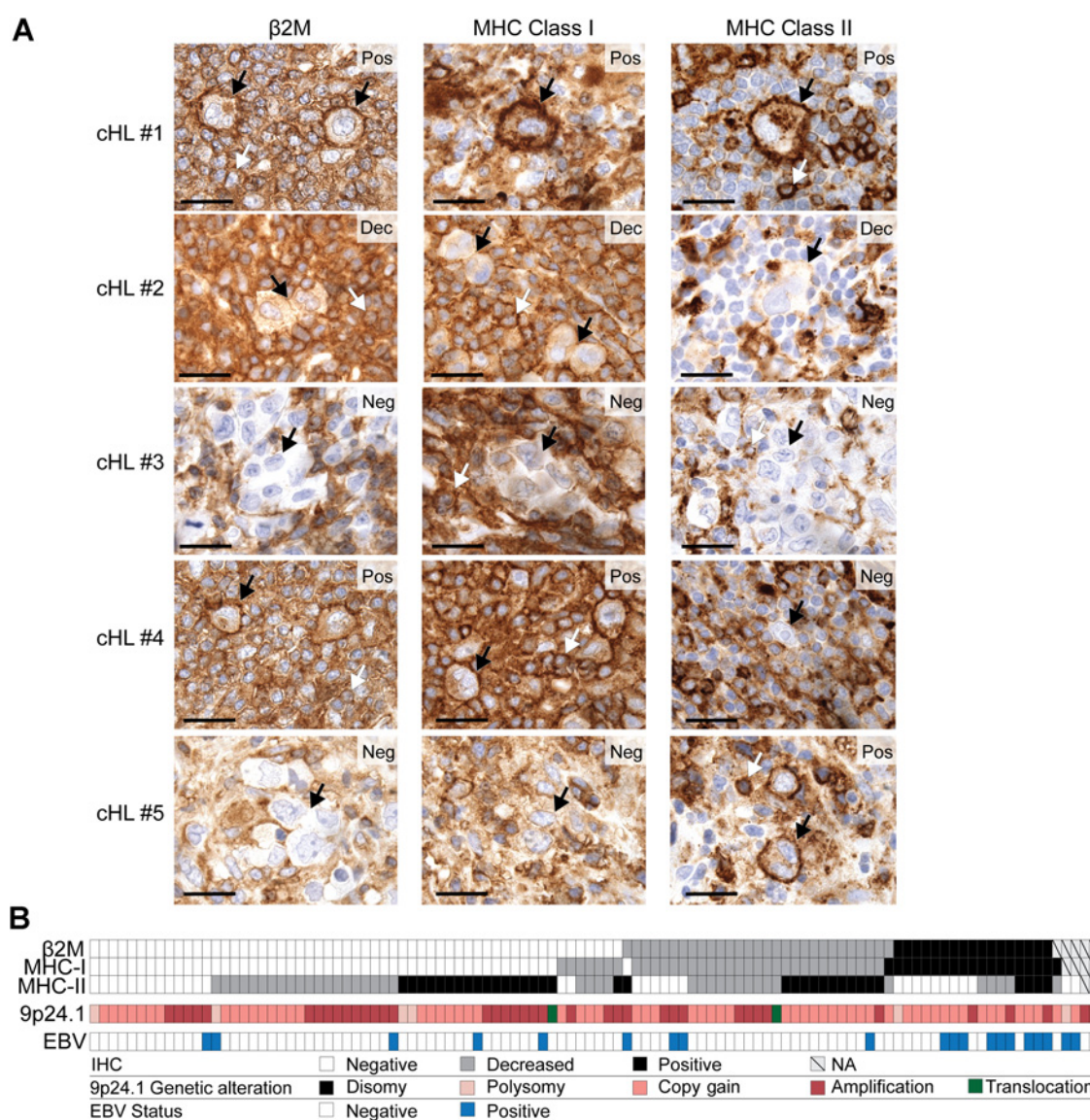
PFS, the time from diagnosis until first progression or death, censored at the time last known alive and progression free, was determined by the method of Kaplan and Meier and compared using log-rank tests. PFS is a preferred metric of response to first-line (induction) therapy, rather than overall survival, as patients who fail induction therapy routinely undergo salvage high-dose chemotherapy and stem cell rescue, a treatment that is curative in a subset of patients. Proportional hazards regression with Firth penalized likelihood were fit using disease stage, *PD-L1*/*PD-L2* amplification, and  $\beta_2$ M, MHC class I, and MHC class II expression. Cox proportional hazards models were compared using likelihood ratio tests, and Wald *P* values were reported for covariates. Associations between continuous, nominal, and ordinal variables were assessed using Wilcoxon rank-sum, Fisher exact, and Kruskal-Wallis tests, respectively. All *P* values were two-sided; values  $\leq 0.05$  were considered statistically significant.

## Results

### Expression of $\beta_2$ M, MHC class I, and MHC class II

We optimized the immunohistochemical staining and scoring methods for  $\beta_2$ M, MHC class I, and MHC class II on a small series of FFPE diagnostic tissue biopsies from patients with cHL (Supplementary Fig. S1). We next evaluated HRS cell expression of  $\beta_2$ M, MHC class I, and MHC class II in diagnostic biopsy specimens from 108 patients with cHL who received uniform treatment and had long-term follow-up (median, 9 years) and previously characterized 9p24.1/*PD-L1*/*PD-L2* alterations (4). Each cHL was classified as positive, decreased, negative, or not assessable for HRS cell membrane expression of  $\beta_2$ M, MHC class I, and MHC class II. In each case, the HRS cell membrane expression was compared with that of nonmalignant cells within the same tissue section. Representative examples are shown in Fig. 1A. Cases included those with positive HRS cell membrane expression of  $\beta_2$ M, MHC class I, and MHC class II (case 1, Fig. 1A); decreased HRS cell membrane expression of  $\beta_2$ M, MHC class I, and MHC class II (case 2, Fig. 1A); and no HRS cell membrane expression of the three markers (case 3, Fig. 1A). Additional cases were positive for  $\beta_2$ M and MHC class I and negative for MHC class II (case 4, Fig. 1A), or negative for  $\beta_2$ M and MHC class I but positive for MHC class II (case 5, Fig. 1A).

The patterns of  $\beta_2$ M, MHC class I, and MHC class II expression in the cHL series are summarized in Fig. 1B. For  $\beta_2$ M, HRS cells were positive in 16% (17/108), decreased in 27% (29/108), negative in 53% (57/108), and unevaluable in 5% (5/108) of cases (Fig. 1B). For MHC class I, HRS cells were positive in 18% (19/108), decreased in 31% (34/108), negative in 47% (51/108), and unevaluable in 4% (4/108) of cases (Fig. 1B). The association between categories of cell-surface  $\beta_2$ M and MHC class I expression (positive, decreased, or absent) by HRS cells was highly significant across cases (Fig. 1B; *P* < 0.001), suggesting that  $\beta_2$ M alterations might be a structural basis for MHC class I loss (2). Overall, HRS



**Figure 1.**  $\beta_2M$ , MHC class I, and MHC class II expression in cHL patients. **A**,  $\beta_2M$ , MHC class I, and MHC class II immunohistochemical staining in 5 representative cHL patients: #1, positive for all markers (Pos); #2, decreased for all markers (Dec); #3, negative for all markers (Neg); #4, positive for  $\beta_2M$  and MHC class I, negative for MHC class II; and #5: negative for  $\beta_2M$  and MHC class I, positive for MHC class II. Black arrow, individual HRS cells; white arrows, expression on surrounding, nonmalignant inflammatory cells. Scale bar, 50  $\mu m$ . **B**, heatmap representing the distribution of  $\beta_2M$ , MHC class I (MHC-I), and MHC class II (MHC-II) expression in the 108 cHL patients. White, negative; gray, decreased; black, positive; and hatched, not assessable (NA). 9p24.1 genetic alterations (light pink, polysomy; medium red, copy gain; dark red, amplification; green, translocation and EBV status; white, negative; blue, positive) are depicted below.

cells in less than 20% of the cases showed positive membrane  $\beta_2M$  or MHC class I staining.

MHC class II expression on HRS cells was positive in 31% (34/108), decreased in 37% (40/108), negative in 30% (32/108), and unevaluable in 2% (2/108) of cases (Fig. 1B). Among the cHLs that were positive for cell-surface MHC class II, only 12% (4/34 cases) were also positive for  $\beta_2M$ /MHC class I; the remainder had decreased/negative  $\beta_2M$ /MHC class I expression [88% (30/34 cases); Fig. 1B]. Consistent with these findings, MHC class II expression was not associated with either  $\beta_2M$  or MHC class I expression (Fig. 1B;  $P = 0.47$  and  $P = 0.21$ , respectively).

### Clinical and biological factors and MHC class I and II expression

We next examined the relationship between specific clinical features and  $\beta_2M$ , MHC class I, and MHC class II HRS cell expression in the patients in whom all three antigen presentation pathway components were evaluable ( $n = 103$ , Table 1). In this series, there was no significant association between age, stage, B symptoms, or bulky disease and altered  $\beta_2M$ , MHC class I, or MHC class II expression (Table 1). In contrast, there were significant associations between the mixed cellularity cHL (MCHL) subtype and positive  $\beta_2M$  and MHC class I expression, and

**Table 1.** Clinical factors

Characteristics		All (n = 103)	β <sub>2</sub> M		P	MHC class I			MHC class II		
			Dec/Neg (n = 86)	Pos (n = 17)		Dec/Neg (n = 85)	Pos (n = 18)	P	Dec/Neg (n = 69)	Pos (n = 34)	P
Age, y	Median (range)	30 (18-69)	29 (18-69)	35 (19-66)	0.13	29 (18-69)	36 (19-66)	0.10	29 (18-69)	31 (18-62)	0.74
Males	n (%)	45	34 (76)	11 (24)	0.07	33 (73)	12 (27)	0.04	31 (69)	14 (31)	0.83
Stage	n (%)				0.18			0.32			0.45
	ES-F	31	23 (74)	8 (26)		23 (74)	8 (26)		21 (68)	10 (32)	
	ES-U	39	34 (87)	5 (13)		34 (87)	5 (13)		23 (59)	16 (41)	
	AS	33	29 (88)	4 (12)		28 (85)	6 (15)		25 (76)	8 (24)	
B symptoms	n (%)	37	33 (89)	4 (11)	0.28	32 (86)	5 (14)	0.59	26 (70)	11 (30)	0.67
Bulky disease	n (%)	48	42 (87)	6 (13)	0.43	42 (87)	6 (13)	0.30	32 (67)	16 (33)	>0.99
Histologic subtype	n (%)				<0.001			<0.001			0.03
	Nodular sclerosis	91	81 (89)	10 (11)		80 (88)	11 (12)		59 (65)	32 (35)	
	Mixed cellularity	9	3 (33)	6 (67)		3 (33)	6 (67)		9 (100)	0 (0)	
	cHL - nos	3	2 (67)	1 (33)		2 (67)	1 (33)		1 (33)	2 (67)	
EBV	n (%)				<0.001			<0.001			0.59
	Negative	85	77 (91)	8 (9)		76 (89)	9 (11)		58 (68)	27 (32)	
	Positive	18	9 (50)	9 (50)		9 (50)	9 (50)		11 (61)	7 (39)	

Abbreviations: AS, advanced stage; cHL - nos, classical Hodgkin lymphoma - not otherwise specified; Dec, decreased; ES-F, early-stage favorable; ES-U, early-stage unfavorable; Neg, negative; Pos, positive.

between Epstein-Barr virus-positive (EBV<sup>+</sup>) cHL and positive β<sub>2</sub>M and MHC class I expression (*P* < 0.001, Table 1), consistent with previous reports (9–12).

**Association of outcome with β<sub>2</sub>M, MHC class I, and MHC class II expression**

We next evaluated a potential association between β<sub>2</sub>M, MHC class I, and MHC class II expression and outcome (PFS) in this series of uniformly treated cHL patients with long-term follow-up (Fig. 2). PFS was comparable in patients whose HRS cells had decreased or negative β<sub>2</sub>M expression (Fig. 2A, left). However, patients whose HRS cells had decreased/negative β<sub>2</sub>M expression had significantly shorter PFS than those whose HRS cells were positive for β<sub>2</sub>M (*P* = 0.037; Fig. 2B, left). Similarly, PFS was comparable in patients with decreased or negative MHC class I expression in HRS cells (Fig. 2A, middle) but significantly shorter than that of patients with positive MHC class I expression in HRS cells (*P* = 0.031; Fig. 2B, middle). Consistent with these results, reduced (decreased/negative) expression of β<sub>2</sub>M and MHC class I had adverse prognostic significance in univariate models (Table 2, *P* = 0.02). MHC class II expression by HRS cells was not significantly associated with PFS in these patients (*P* = 0.60; Fig. 2; Table 2). In addition, neither the cHL subtype [MCHL vs. nodular sclerosis Hodgkin lymphoma (NSHL)] nor EBV status was associated with PFS (not shown).

**Antigen presentation pathway components and 9p24.1/PD-L1/PD-L2 status**

In this series of cHL patients, we found that 9p24.1/PD-L1/PD-L2 amplification was associated with advanced stage disease and inferior PFS (4). Given these findings, we next evaluated the prognostic significance of PD-L1/PD-L2 amplification in patients with defined HRS cell expression of β<sub>2</sub>M/MHC class I (Fig. 2C). In patients with positive HRS cell expression of β<sub>2</sub>M and MHC class I, PFS was not affected by 9p24.1 amplification, although only 3 patients had 9p24.1 amplification and positive β<sub>2</sub>M and MHC class I (Fig. 2C, left and middle). In marked contrast, 9p24.1 amplification adversely impacted PFS in patients whose HRS cells had decreased/negative β<sub>2</sub>M/MHC class I expression (Fig. 2C, left

and middle). Importantly, these results were highly significant despite the small numbers of 9p24.1, β<sub>2</sub>M-positive, and MHC class I-positive patients (*P* = 0.014 and *P* = 0.013, respectively).

In multivariable models of (i) decreased/negative MHC class I expression and 9p24.1 amplification or (ii) decreased/negative MHC class I expression and advanced stage disease, both features retained adverse prognostic significance (Table 2). When all three features were included in the multivariable model, decreased/negative expression of MHC class I still retained adverse prognostic significance (Table 2, *P* = 0.05). Similar results were obtained in Cox models containing β<sub>2</sub>M rather than MHC class I (Supplementary Table S2).

**Discussion**

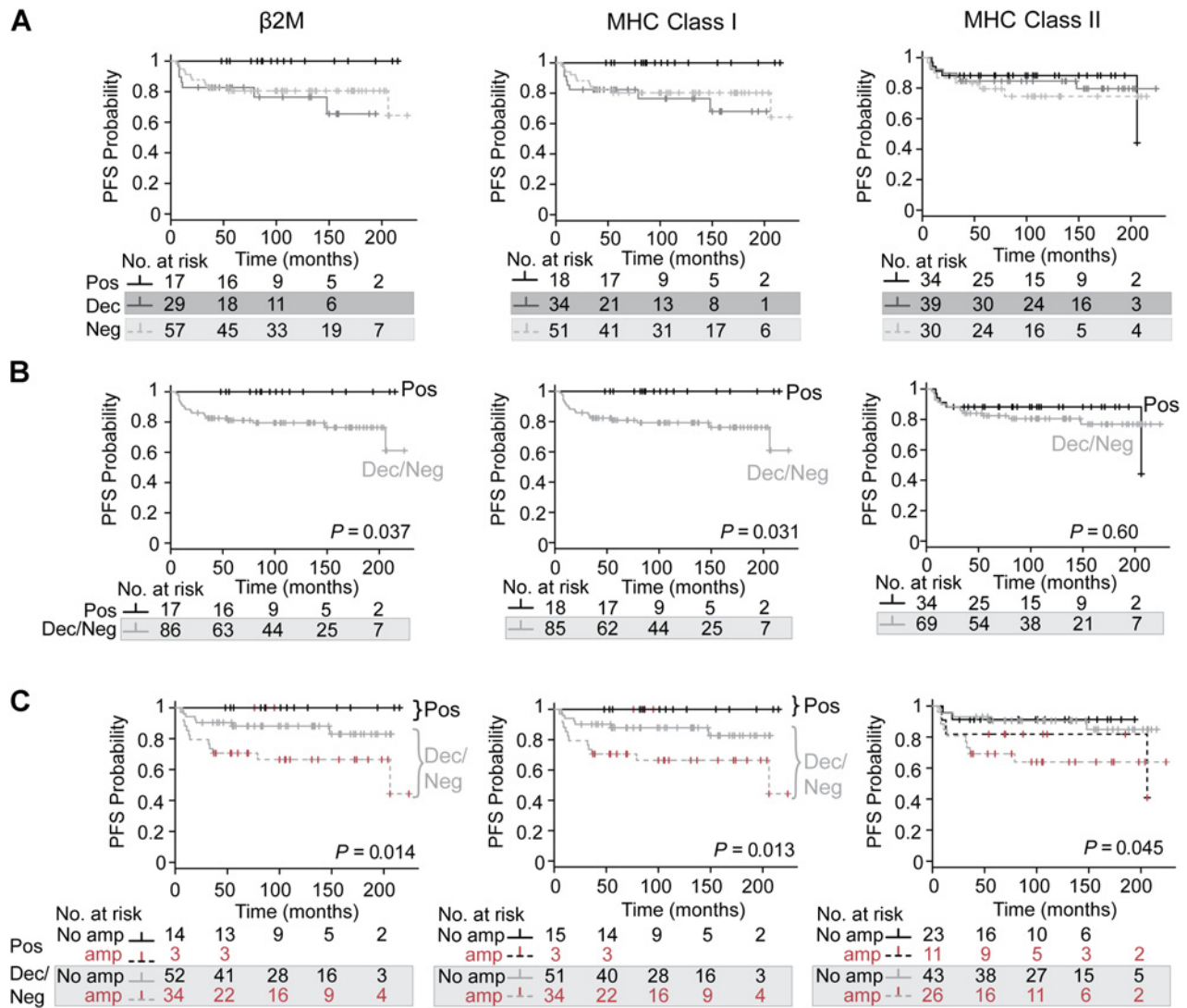
In this analysis, we found that (i) decreased or absent β<sub>2</sub>M and MHC class I expression and decreased or absent MHC class II expression on HRS cells occur in approximately 80% and 70% of cHL cases, respectively; (ii) decreased/absent β<sub>2</sub>M/MHC class I expression, but not decreased/absent MHC class II expression, is associated with shorter PFS; and (iii) the prognostic value of decreased/absent β<sub>2</sub>M/MHC class I expression is independent of PD-L1/PD-L2 amplification and advanced stage disease.

The B2M subunit is required for assembly of MHC class I on the cell surface of nucleated cells. As expected, we observed a high concordance between β<sub>2</sub>M and MHC class I IHC scores. For cases of cHL categorized as negative for β<sub>2</sub>M expression, we found that loss of β<sub>2</sub>M from the HRS cells was complete. In contrast, HRS cells in certain cases exhibited cytoplasmic expression of MHC class I in the absence of membranous staining (Fig. 1, cases #3 and #5 vs. case #4). These data suggest that loss of β<sub>2</sub>M expression is the predominant mechanism for deficient cell-surface MHC class I protein expression in cHL, as previously suggested by genetic studies (2).

In contrast to prior reports in cHL, we observed unequivocally reduced, but not completely absent, β<sub>2</sub>M, MHC class I, and class II membrane expression on HRS cells relative to normal, surrounding inflammatory cells in a significant subset (30%–40%) of cases (2, 13). We used a 3-tier scoring system, positive, decreased, and

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**Figure 2.** PFS by  $\beta_2M$ , MHC class I, and MHC class II expression in the cHL cohort. **A**, PFS for patients with positive (Pos), decreased (Dec), and negative (Neg) HRS cell membrane expression of  $\beta_2M$  (left), MHC class I (middle), and MHC class II (right). **B**, PFS for patients with positive versus decreased/negative HRS cell expression of  $\beta_2M$  (left), MHC class I (middle), and MHC class II (right). **C**, PFS for patients whose HRS cells have positive or decreased/negative  $\beta_2M$  (left), MHC class I (middle), and MHC class II (right) expression in the presence or absence of *PD-L1/PD-L2* amplification (amp).

negative, to capture the heterogeneity of  $\beta_2M$ /MHC class I and MHC class II protein expression. Cases with reduced, but not completely absent, expression of the  $\beta_2M$ /MHC class I, and MHC class II proteins may have single-allele loss or a heterozygous-inactivating mutation in genes directly encoding these proteins (such as single-allele inactivation of *B2M* or single-copy loss of the HLA locus) or alterations in genes encoding critical transcriptional regulators (such as *NLR5* and *CIITA*; refs. 3, 14, 15). We also observed cases, classified as decreased, with positive expression in only a subset of HRS cells, suggesting that subclones with distinct characteristics can exist within a single case.

In contrast to a recent study (2), we found that cHL patients with decreased or negative  $\beta_2M$  expression have inferior outcomes (Fig. 2; Table 2). Unlike the prior study, patients in this series

received uniform treatment at a single institution and had pre-specified clinical follow-up for a median of 9 years. In further contrast, this series includes a representative mix of early- and advanced stage disease and NSHL and MCHL. As previously reported for cHL, we found that patients with advanced stage disease had inferior PFS, and patients with MCHL and/or EBV<sup>+</sup> cHL had significantly higher  $\beta_2M$ /MHC class I expression on HRS cells (4, 9–12). Given these characteristics, we believe that the current series provides a comprehensive framework for analyzing the prognostic significance of  $\beta_2M$ , MHC class I, and MHC class II expression in newly diagnosed cHL patients treated with standard induction therapy. In addition, these data highlight the likely biological importance MHC class I-mediated antigen presentation by HRS cells to cytotoxic T cells for optimal clinical response

**Table 2.** Outcome (PFS) - Cox univariate and multivariable models

Univariate models	HR	SE	P
$\beta_2$ M			
$\beta_2$ M decreased/negative	9.2	1.47	0.02
MHC class I			
MHC class I decreased/negative	9.7	1.47	0.02
MHC class II			
MHC class II decreased/negative	1.2	0.52	0.66
9p24.1 genetic status			
9p24.1 amplification	3.3	0.47	0.01
Clinical factors			
Advanced stage	3.1	0.46	0.01
Multivariate models	HR	SE	P
MHC class I decreased/negative, 9p24.1 amplification			
MHC class I decreased/negative	7.3	1.48	0.05
9p24.1 amplification	2.7	0.47	0.03
MHC class I decreased/negative, advanced stage			
MHC class I decreased/negative	8.9	1.47	0.03
Advanced stage	2.9	0.47	0.02
MHC class I decreased/negative, advanced stage, 9p24.1 amplification			
MHC class I decreased/negative	7.4	1.48	0.05
Advanced stage	2.5	0.47	0.04
9p24.1 amplification	2.3	0.48	0.07

Abbreviations: HR, hazard ratio; SE, standard error.

to nonimmune therapy. That MHC class I expression is associated with a more favorable outcome to standard therapy in cHL is consistent with the findings in multiple other tumor types (16–19).

The paucity of  $\beta_2$ M/MHC class I expression on HRS cells also prompts speculation regarding additional mechanisms of action of PD-1 blockade in cHL. In other tumor types, clinical responses to standard chemotherapy and immunotherapies, including checkpoint blockade, have been associated with the presence of CD8<sup>+</sup> T cells within the tumor microenvironment, underscoring the importance of  $\beta_2$ M/MHC class I-mediated antigen presentation by malignant cells (16, 20–22). Patients with relapsed/refractory cHL who are treated with PD-1-blocking antibodies have response rates of 65% to 87% and long-lasting remissions. Yet, the complete loss of MHC class I and  $\beta_2$ M expression by HRS

cells in approximately one half of cHLs suggests that CD8<sup>+</sup> cytotoxic T cell-mediated killing of HRS cells may not be the only mechanism of antitumor immunity augmented by PD-1 blockade. Given our findings, the significance of impaired antigen presentation by HRS cells on the quality and durability of clinical responses to PD-1 inhibitors will be of great interest.

### Disclosure of Potential Conflicts of Interest

M.A. Shipp reports receiving a commercial research grant from and is a consultant/advisory board member for Bristol-Myers Squibb. S.J. Rodig reports receiving a commercial research grant from and has received speakers bureau honoraria from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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### References

- Kuppers R. The biology of Hodgkin's lymphoma. *Nat Rev Cancer* 2009;9:15–27.
- Reichel J, Chadburn A, Rubinstein PG, Giulino-Roth L, Tam W, Liu Y, et al. Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells. *Blood* 2015;125:1061–72.
- Steidl C, Shah SP, Woolcock BW, Rui L, Kawahara M, Farinha P, et al. MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. *Nature* 2011;471:377–81.
- Roemer MG, Advani RH, Ligon AH, Natkunam Y, Redd RA, Homer H, et al. PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. *J Clin Oncol* 2016;34:2690–7.
- Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 2015;372:311–9.
- Armand P, Shipp MA, Ribrag V, Michot JM, Zinzani PL, Kuruvilla J, et al. Programmed death-1 blockade with pembrolizumab in patients with classical Hodgkin lymphoma after brentuximab vedotin failure. *J Clin Oncol*. 2016 Jun 27. [Epub ahead of print].
- Younes A, Santoro A, Shipp MA, Zinzani PL, Timmerman JM, Ansell S, et al. Nivolumab for classical Hodgkin lymphoma after autologous stem-cell transplantation and brentuximab vedotin failure: a prospective phase 2 multi-cohort study. *Lancet Oncol* 2016;17:1283–94.
- Chapuy B, Roemer MG, Stewart C, Tan Y, Abo RP, Zhang L, et al. Targetable genetic features of primary testicular and primary central nervous system lymphomas. *Blood* 2016;127:869–81.
- Diepstra A, Niens M, Vellenga E, van Imhoff GW, Nolte IM, Schaapveld M, et al. Association with HLA class I in Epstein-Barr-virus-positive and with HLA class III in Epstein-Barr-virus-negative Hodgkin's lymphoma. *Lancet* 2005;365:2216–24.
- Lee SP, Constantinou CM, Thomas WA, Croom-Carter D, Blake NW, Murray PG, et al. Antigen presenting phenotype of Hodgkin Reed-Sternberg cells: analysis of the HLA class I processing pathway and the effects of interleukin-10 on Epstein-Barr virus-specific cytotoxic T-cell recognition. *Blood* 1998;92:1020–30.
- Oudejans JJ, Jiwa NM, Kummer JA, Horstman A, Vos W, Baak JP, et al. Analysis of major histocompatibility complex class I expression on Reed-Sternberg cells in relation to the cytotoxic T-cell response in Epstein-Barr virus-positive and -negative Hodgkin's disease. *Blood* 1996;87:3844–51.
- Huang X, Kushkhar K, Nolte I, Kooistra W, Visser L, Bouwman I, et al. HLA associations in classical Hodgkin lymphoma: EBV status matters. *PLoS One* 2012;7:e39986.
- Diepstra A, van Imhoff GW, Karim-Kos HE, van den Berg A, te Meerman GJ, Niens M, et al. HLA class II expression by Hodgkin Reed-Sternberg cells is an independent prognostic factor in classical Hodgkin's lymphoma. *J Clin Oncol* 2007;25:3101–8.

14. Mottok A, Woolcock B, Chan FC, Tong KM, Chong L, Farinha P, et al. Genomic alterations in CIITA are frequent in primary mediastinal large B cell lymphoma and are associated with diminished MHC class II expression. *Cell Rep* 2015;13:1418–31.
15. Yoshihama S, Roszik J, Downs I, Meissner TB, Vijayan S, Chapuy B, et al. NLRC5/MHC class I transactivator is a target for immune evasion in cancer. *Proc Natl Acad Sci U S A* 2016;113:5999–6004.
16. de Kruijf EM, van Nes JG, Sajet A, Tummers QR, Putter H, Osanto S, et al. The predictive value of HLA class I tumor cell expression and presence of intratumoral Tregs for chemotherapy in patients with early breast cancer. *Clin Cancer Res* 2010;16:1272–80.
17. Jordanova ES, Gorter A, Ayachi O, Prins F, Durrant LG, Kenter GG, et al. Human leukocyte antigen class I, MHC class I chain-related molecule A, and CD8+/regulatory T-cell ratio: which variable determines survival of cervical cancer patients? *Clin Cancer Res* 2008;14:2028–35.
18. Simpson JA, Al-Attar A, Watson NF, Scholefield JH, Ilyas M, Durrant LG. Intratumoral T cell infiltration, MHC class I and STAT1 as biomarkers of good prognosis in colorectal cancer. *Gut* 2010;59:926–33.
19. Turcotte S, Katz SC, Shia J, Jarnagin WR, Kingham TP, Allen PJ, et al. Tumor MHC class I expression improves the prognostic value of T-cell density in resected colorectal liver metastases. *Cancer Immunol Res* 2014;2:530–7.
20. Tumeq PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
21. Restifo NP, Marincola FM, Kawakami Y, Taubenberger J, Yannelli JR, Rosenberg SA. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J Natl Cancer Inst* 1996;88:100–8.
22. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 2016;375:819–29.