



Patterns of immune checkpoint protein expression in MYC-overexpressing aggressive B-cell non-Hodgkin lymphomas

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Abstract

Given the poor prognosis of MYC-overexpressing diffuse large B cell lymphoma (DLBCL) and B cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma/high grade B cell lymphoma (BCLU/HGBL), and preclinical data suggesting that MYC may regulate the antitumor immune response, we sought to characterize expression of immune checkpoint proteins on tumor tissue from patients diagnosed with these lymphomas. Immunohistochemical staining for immune checkpoint protein expression was applied to 56 cases of MYC-overexpressing DLBCL and BCLU/HGBL, 35 of which also harbored MYC rearrangement (MYC-R). Analysis revealed both frequent overexpression of immune checkpoint proteins as well as differences in overexpression patterns based upon MYC-R status, with MYC-R cases more likely to overexpress PD-L1 and PD-1 in the tumor microenvironment (50 vs. 15%, $p=0.02$ and 32 vs. 5%, $p=0.02$, respectively) but less likely to overexpress CTLA-4 and CD80 on tumor cells (34 vs. 71%, $p=0.01$ and 34 vs. 81%, $p=0.001$, respectively), as compared to cases without MYC-R. These data may suggest a biologic rationale for investigation of the effect of checkpoint inhibitor therapies in these subgroups of MYC-overexpressing DLBCL and BCLU/HGBL.

Keywords MYC · Diffuse large B cell lymphoma · High grade B cell lymphoma · PD-1 · PD-L1

Introduction

Patients diagnosed with diffuse large B cell lymphoma (DLBCL) and the recently re-classified histology of B cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma/high grade B cell lymphoma (BCLU/HGBL) experience variable outcomes following receipt of the standard front-line immunochemotherapy regimen of rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). One of the more well-characterized poor prognosis features of these diseases at initial diagnosis is the presence of MYC rearrangement (MYC-R) [1, 2] as well as overexpression of MYC protein [3, 4], independent of MYC-R status, and testing for these MYC alterations is frequently performed in clinical practice.

Given a high likelihood that patients with these MYC-altered lymphomas will develop chemorefractory disease, treatment with non-cytotoxic agents may be beneficial.

Immune checkpoint inhibitors have emerged as effective therapies in solid tumor malignancies as well as classical Hodgkin lymphoma, and has led to approval of inhibitors of programmed cell death protein 1 (PD-1), programmed-death ligand 1 (PD-L1) and CTLA-4 by the United States Food and Drug Administration (US FDA). In DLBCL, PD-L1 overexpression is reported to be present on approximately 10–50% of cases [5, 6], with variations in reported frequency potentially due to overexpression cutoffs, inter-laboratory differences in IHC sensitivity for these markers, patient ethnicity and receipt of prior therapy. PD-L1 expression is associated with both non-germinal center cell of origin (non-GCB COO) and unfavorable prognosis following receipt of first-line R-CHOP in DLBCL patients [6].

Preclinical data have demonstrated that PD-L1 expression on lymphoma cells may be driven by activation of MYC [7], although tumors from patients with previously untreated DLBCL or BCLU/HGBL harboring MYC-R [8] or expressing MYC protein [5] may demonstrate lower

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levels of PD-L1 expression on tumor cells and tumor microenvironment (TME) cells, respectively, than those without MYC alterations. There are no published data on expression patterns of other immune checkpoint proteins DLBCL and BCLU/HGBL, although it has been hypothesized that MYC may regulate their expression [7].

In light of the above findings, as well as the clinical availability of checkpoint inhibitor therapies, we sought to determine the prevalence of immune checkpoint protein overexpression on cases of DLBCL and BCLU/HGBL with MYC overexpression and correlate this with MYC-R status, as well as clinical outcomes for previously untreated patients following receipt of front-line immunochemotherapy.

Materials and methods

Cases were identified via review of an internal database of DLBCL and BCLU/HGBL specimens which were reviewed at the University of Pennsylvania from 2009–2018. Those reported to demonstrate increased expression of MYC protein by IHC ($\geq 40\%$) by clinical hematopathology review were selected from the database and tumor tissue was obtained from these cases when available. Cases of primary central nervous system lymphoma, post-transplant lymphoproliferative disorder and from patients with human immunodeficiency virus positivity were excluded. Cutoffs for overexpression of MYC ($\geq 40\%$) and BCL2 ($\geq 50\%$) [9], PD-L1 on tumor cells ($\geq 30\%$) [6], PD-L1 on TME cells ($\geq 20\%$) [6] and PD-1 on TME cells ($\geq 30\%$) [10] were as previously published for DLBCL; a cutoff of $\geq 30\%$ to denote overexpression was used for all other proteins. The presence or absence of MYC-R was detected by fluorescence in situ hybridization with MYC breakapart probe. Cases were reviewed by two expert hematopathologists (GCC and MAW). Relapse free survival (RFS) was defined as time from the start of therapy to disease progression or last follow-up in remission. Overall survival (OS) was defined as time from the start of therapy to death from any cause or last follow-up while alive. Disease progression was defined by clinical radiology report as per the Revised Response Criteria for Malignant Lymphoma [11]. Categorical data were analyzed by Fisher's exact test. Survival analysis was performed with the log-rank test and the impact of categorical variables on both survival and achievement of response was analyzed using Cox proportional-hazard regressions. Statistical significance was defined as a two-tailed p value of < 0.05 for all analyses. Data were censored on November 1, 2019. This protocol was approved by the Institutional Review Board of the University of Pennsylvania.

Results

Database query yielded 197 cases of DLBCL or BCLU/HGBL with MYC overexpression. Further stratification and inclusion/exclusion of cases is depicted in Fig. 1. In total, tissue was available for analysis from 56/197 (40%) cases and was similar for patients with (39/100, 39%) and without (40/97, 41%) MYC-R. Ultimately 35 cases from 31 patients with MYC-R and 21 cases from 21 patients without MYC-R were included in the analysis. The proportion of overexpression of immune checkpoint proteins from all cases, as well as the subset from previously untreated patients, are listed in Table 1. Overexpression of both PD-L1 and PD-1 in the TME was more frequently seen in cases with MYC-R than those without (50 vs. 15%, $p = 0.02$ and 32 vs. 5%, $p = 0.02$, respectively) and overexpression of CTLA-4 and CD80 on tumor cells less frequently seen in cases with MYC-R than those without (34 vs. 71%, $p = 0.01$ and 34 vs. 81%, $p = 0.001$, respectively). In addition, analysis for concurrent overexpression of proteins relevant to the PD-1 and CTLA-4 pathways demonstrated statistically significant co-overexpression of PD-L1 on tumor cells and pSTAT3 ($p = 0.001$), PD-L1 and PD-1 both on TME cells ($p < 0.001$) as well as CD80 and CTLA-4 ($p = 0.009$). While a significantly higher percentage of MYC-R specimens were classified as GCB COO by Hans algorithm [12] (80 vs. 52%, $p = 0.04$), there were no statistically significant differences in immune checkpoint protein overexpression patterns when comparing cohorts by COO. Immune checkpoint protein overexpression patterns were similar when restricting this analysis to cases from previously untreated patients.

Analysis of clinical outcomes was performed for previously untreated patients ($n = 37$). Relevant clinicopathologic baseline characteristics reported included female sex in $n = 14$ (38%), age > 60 years in $n = 19$ (51%), elevated LDH in $n = 31$ (91%), stage 3–4 disease in $n = 28$ (76%), Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2 in $n = 4$ (11%), extranodal disease in $n = 25$ (68%), Revised International Prognostic Index (R-IPI) score ≥ 3 in $n = 20$ (59%), GCB COO in $n = 30$ (70%) and Ki67 $\geq 90\%$ in $n = 16$ (46%) patients. Of MYC-R patients ($n = 24$), $n = 12$ (50%) were classified as double hit lymphoma (7 with *MYC/BCL2*, 2 with *MYC/BCL6* and 3 with *MYC/BCL2/BCL6* rearrangements) and $n = 19$ (83%) of non-double hit lymphoma patients (including those with sole MYC-R) were classified as double expressor lymphoma (overexpression of both MYC and BCL2 proteins).

Front-line therapy was R-CHOP in $n = 13$ (35%), intensive immunochemotherapy in $n = 25$ (65%) with nearly all of these patients receiving dose-adjusted etoposide,

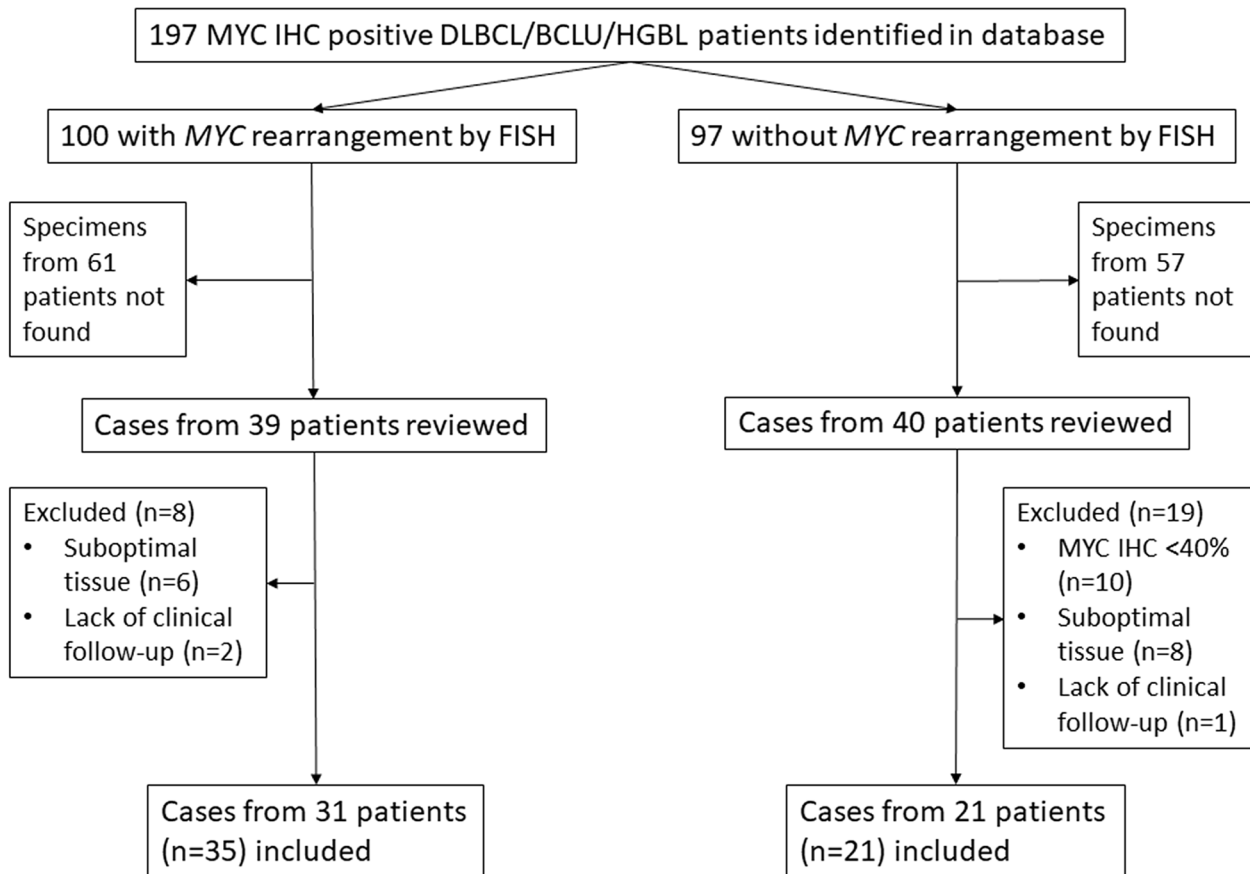


Fig. 1 Case inclusion/exclusion

prednisone, vincristine, cyclophosphamide, etoposide with rituximab (DA-EPOCH-R), and other regimens in $n=3$ (10%) cases. With a median length of follow-up of 40.1 months, 2 year RFS was 57% (95% confidence interval [CI] 38–72%) and 2 year OS 58% (95% CI 40–73%) as shown in Fig. 2. Accurate univariate and/or multivariate analysis for survival based upon baseline clinicopathologic characteristics could not be performed due to the small sample size of patients.

Discussion

Our data show relatively high rates of immune checkpoint protein overexpression in cases of MYC-overexpressing DLBCL and BCLU/HGBL, with statistically significant differences based upon MYC-R status, with MYC-R specimens more likely to overexpress both PD-L1 and PD-1 in the TME and non-MYC-R specimens CD80 and CTLA-4. RFS and OS at 2 years following receipt of front-line immunochemotherapy were as expected based upon prior analyses of patients with MYC-overexpressing DLBCL and BCLU/

HGBL with or without MYC-R treated with intensive front-line immunochemotherapy in the majority of cases [13], suggesting that our cohort is representative of the larger population of these patients encountered in clinical practice.

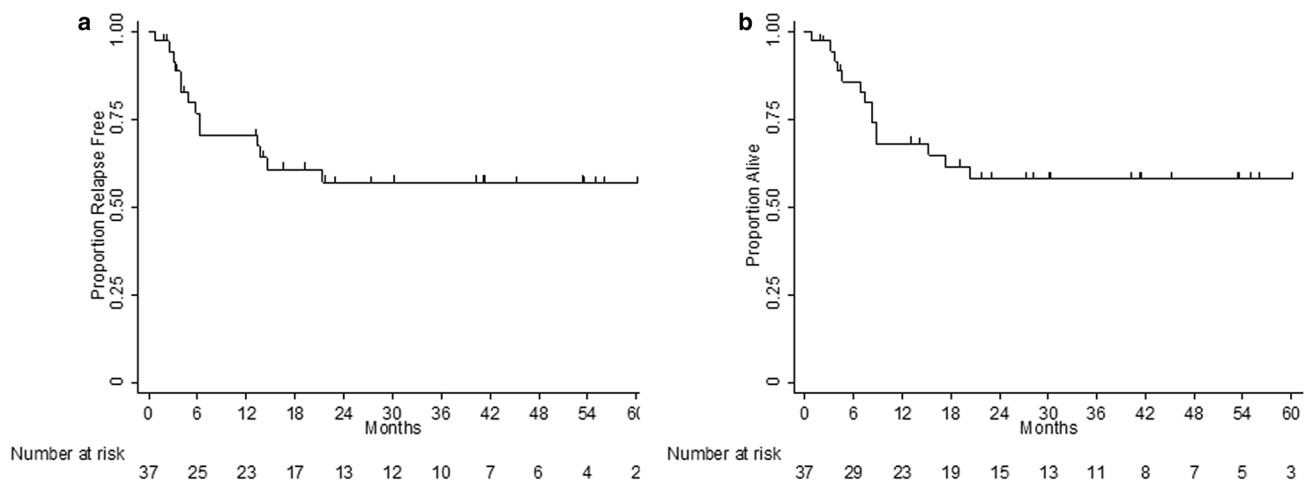
Our data agree with others demonstrating a low frequency of PD-L1 overexpression in previously-untreated MYC-altered DLBCL and BCLU/HGBL [5, 8], which we hypothesize is due to the relative lack of pSTAT3 expression, as this appears to be required for PD-L1 expression in T-cell non-Hodgkin lymphomas [14, 15]. Interestingly, all three cases with pSTAT3 overexpression also demonstrated PD-L1 overexpression. Also of note is the fact that PD-L1 overexpression on tumor cells was only detected in previously-treated cases ($n=6$, 31%), including two cases with both pre- and post-treatment biopsies, suggesting that overexpression of PD-L1 on tumor cells could serve as a mechanism of resistance to front-line immunochemotherapy in these lymphomas.

As seen in our cohort, discordance of the degree of PD-L1 overexpression on tumor vs. TME cells has been previously reported in clinical trials evaluating the therapeutic efficacy of PD-L1 inhibitor therapy in non-small cell lung

Table 1 Proportion of cases with increased immune checkpoint protein expression by immunohistochemical staining from (A) all patients regardless of treatment status and (B) previously untreated patients only

A	All cases (n = 56)	No MYC-R (n = 21)	MYC-R (n = 35)	p
PD-L1	6 (11%)	2 (10%)	4 (11%)	1
PD-L1 tumor microenvironment	20 (37%)	3 (15%)	17 (50%)	0.018
PD-1 tumor microenvironment	12 (22%)	1 (5%)	11 (32%)	0.019
pSTAT3	3 (5%)	1 (5%)	2 (6%)	1
pSTAT5	41 (75%)	12 (60%)	29 (83%)	0.11
CD80	29 (52%)	17 (81%)	12 (34%)	0.001
CTLA-4	27 (48%)	15 (71%)	12 (34%)	0.012
CD86	31 (56%)	8 (40%)	23 (66%)	0.091
pERK	2 (4%)	0	2 (6%)	0.52
B	All cases (n = 37)	No MYC-R (n = 13)	MYC-R (n = 24)	p
PD-L1	0	0	0	–
PD-L1 tumor microenvironment	14 (38%)	2 (15%)	12 (50%)	0.07
PD-1 tumor microenvironment	9 (25%)	1 (7%)	8 (35%)	0.11
pSTAT3	0	0	0	–
pSTAT5	27 (75%)	8 (67%)	19 (79%)	0.44
CD80	16 (43%)	10 (77%)	6 (25%)	0.05
CTLA-4	17 (46%)	9 (69%)	8 (33%)	0.047
CD86	17 (47%)	3 (25%)	14 (58%)	0.08
pERK	1 (3%)	0	1 (4%)	1

All proteins were detected on tumor cells unless otherwise noted

**Fig. 2** **a** Relapse free survival and **b** overall survival of previously untreated patients

cancer (NSCLC) patients, with responses seen in patients overexpressing PD-L1 on either tumor or TME cells [16]. Overexpression of PD-L1 on TME cells, as demonstrated in approximately 1/3 of cases in our series, may be of clinical relevance given that the PD-L1 inhibitor atezolizumab was approved by the US FDA for treatment of metastatic NSCLC and triple negative breast cancer with increased expression of PD-L1 on tumor-infiltrating immune cells based upon efficacy data for these patient populations [17].

Preclinical data have demonstrated that PD-L1 expression is regulated by *MYC* gene function in tumor cells [18] and expression of *MYC* protein is significantly higher in tumors harboring PD-1 positive T cells along with PD-L1 positive, but not PD-L1 negative, tumor cells [19]. However, it is unclear why the subset of cases with *MYC*-R were more likely to overexpress PD-L1 and PD-1 in the TME as compared to cases without *MYC*-R in our series. *MYC* protein expression has been associated with *MYC* mutations [3],

copy number alterations [20] as well as other mechanisms of upregulation [9] independent of MYC-R, and associations between pathways driving MYC protein and immune checkpoint protein expression could be explored further.

Limitations of this analysis include a relatively small sample size, which precludes our ability to determine whether or not survival outcomes were associated with the presence or absence of overexpression of specific immune checkpoint proteins. Additionally, we were unable to obtain tissue for analysis of all cases identified by database query.

In conclusion, these data may support further investigation of immune checkpoint protein regulation/expression as well as the therapeutic efficacy of checkpoint inhibitors in MYC-overexpressing DLBCL and BCLU/HGBL.

Author contributions DJL, SJS, MAW and GCC contributed to the study conception and design. Material preparation, data collection and analysis were performed by DJL, MAW and GCC. The first draft of the manuscript was written by DJL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material Data may be made available if requested.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This protocol was approved by the Institutional Review Board of the University of Pennsylvania.

Consent to participate A waiver of informed consent was granted by the Institutional Review Board of the University of Pennsylvania.

Consent for publication Not applicable.

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