Activation of the STAT3 Signaling Pathway Is Associated With Poor Survival in Diffuse Large B-Cell Lymphoma Treated With R-CHOP

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A B S T R A C T

Purpose

We previously reported that constitutive STAT3 activation is a prominent feature of the activated B-cell subtype of diffuse large B-cell lymphomas (ABC-DLBCL). In this study, we investigated whether STAT3 activation can risk stratify patients with DLBCL.

Patients and Methods

By an immunohistochemical method, we investigated phosphotyrosine STAT3 (PY-STAT3) expression from 185 patients with DLBCL treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone). Cell line-based siRNA experiments were also performed to generate an 11-gene, PY-STAT3 activation signature, which was used to study a previously published cohort of 222 patients with DLBCL. The STAT3 activation status determined by these two methods and by STAT3 mRNA levels were then correlated with survival.

Results

PY-STAT3 was detected in 37% of DLBCL and enriched in ABC-DLBCL cases ($P = .03$). PY-STAT3 positivity significantly correlated with poor overall survival (OS; $P = .01$) and event-free survival (EFS; $P = .006$). Similar observations were made for high levels of STAT3 mRNA. In multivariable analysis, PY-STAT3 status ($P = .02$), International Prognostic Index ($P = .02$), and BCL2 expression ($P = .046$) were independent prognosticators of OS in this cohort. Among the cell-of-origin subgroups, PY-STAT3 was associated with poor EFS among non-germinal center B-cell DLBCL cases only ($P = .027$). Similarly, the 11-gene STAT3 activation signature correlated with poor survival in the entire DLBCL cohort (OS, $P < .001$; EFS, $P < .001$) as well as the ABC-DLBCL subgroup (OS, $P = .029$; EFS, $P = .025$).

Conclusion

STAT3 activation correlated with poor survival in patients with DLBCL treated with R-CHOP, especially those with tumors of the ABC-DLBCL subtype.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) accounts for approximately 30% to 40% of newly diagnosed non-Hodgkin lymphoma.¹ The addition of rituximab (R-) to standard CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy regimens results in improved overall survival (OS) by 10% to 15%.² Nevertheless, a substantial number of patients still die as a result of the disease, highlighting the need for improved DLBCL prognostication and better therapy. DLBCL

is a biologically and clinically heterogeneous disease, which is explained, at least in part, by the diversity in its normal cellular counterparts and transforming pathways.³ On the basis of gene expression similarities to either normal germinal center (GC) B cells or activated peripheral blood B cells, DLBCL can be divided into two main subgroups: germinal center B-cell-like (GCB) DLBCL and activated B-cell-like (ABC) DLBCL.^{4,5} In this cell-of-origin (COO) classification, GCB-DLBCL represents transformed GC centroblasts that are BCL6 high and lack features of B-cell activation. In comparison, ABC-DLBCLs,

likely correspond to activated centrocytes and/or preplasmablasts³ and are characterized by constitutively activated NF- κ B as well as JAK/STAT3 activation in many but not all cases.⁶⁻⁸ It is also well documented that patients with GCB-DLBCL generally have a better prognosis than do patients with ABC-DLBCL in both the CHOP⁹ and R-CHOP eras.¹⁰⁻¹³ It is therefore important to identify new biomarkers that can risk-stratify ABC-DLBCL for the development and application of novel targeted therapies.

In normal cells, STAT3 activation is a transient and tightly controlled process because of rapid activation and feedback inactivation of growth factor/cytokine receptor signaling.¹⁴ In many types of solid tumors, aberrant activation of upstream tyrosine kinases leads to constitutive activation of JAK/STAT3 signaling, which in turn promotes tumor cell growth, survival, angiogenesis, and metastasis.¹⁵ Through inflammatory mediators in the tumor microenvironment, tumor cells with activated STAT3 can also evade immune surveillance by inhibiting antitumor immune responses.¹⁵ In lymphoid malignancies, a pathogenic role of STAT3 has been shown in multiple myeloma, Hodgkin lymphoma, anaplastic large T-cell lymphoma, and, recently, in ABC-DLBCL.^{7,8,16-19} Three mechanisms have been described to account for persistent STAT3 activation in ABC-DLBCL. First, constitutive NF- κ B activation leads to production of interleukin (IL)-6 and IL-10, both of which are STAT3-activating cytokines.⁸ In addition, 29% of ABC-DLBCLs express mutated MYD88 (L265P), which triggers cell signaling along the IRAK1/4-NF- κ B axis as well as the JAK/STAT3 axis.²⁰ Finally, high expression of HDAC3 in ABC-DLBCL promotes STAT3 activity by modulating acetylation and subcellular localization.²¹ An oncogenic role of STAT3 in ABC-DLBCL has been shown by studies using cell culture systems^{8-9,22} and mouse xenograft models.²² However, the prognostic significance of STAT3 activation has not been thoroughly evaluated in patients with DLBCL. In a recent report involving a small cohort of DLBCL cases, strong nuclear staining for STAT3 correlated with poor survival of patients treated with CHOP.²³ Herein, we report a retrospective analysis of a large cohort of patients with DLBCL treated with R-CHOP. This study was designed to test the specific hypothesis that constitutive STAT3 activation can be used as a biomarker for poor prognosis in R-CHOP-treated DLBCL.

PATIENTS AND METHODS

Patient Information

The sources of patient-derived material and data are summarized in the Data Supplement. The primary patient cohort included 309 patients with de novo DLBCL who received R-CHOP treatment. Among these patients, 87 were treated at the University of Nebraska Medical Center (UNMC), whereas the remaining 222 cases were treated at other Lymphoma/Leukemia Molecular Profiling Project (LLMPP)-affiliated institutions.¹³ A subset of the primary cohort ($n = 185$) comprising the 87 UNMC cases and 98 cases from the LLMPP R-CHOP series were studied for PY-STAT3 expression using immunohistochemistry (IHC). Validation cohort 1 is a LLMPP CHOP series with 181 patients¹³ and validation cohort 2 is an R-CHOP cohort consisting of 65 patients with DLBCL treated at the British Columbia Cancer Agency, Vancouver, Canada.²⁴ Clinical features of the patients were retrieved either from the clinical database of Department of Pathology and Microbiology at UNMC or from the Gene Expression Omnibus (GEO) database. This study was approved by the institutional review board of UNMC.

Tissue Microarray and IHC Analysis

Tissue microarray (TMA) construction, IHC procedure, and scoring criteria for CD10, BCL6, Mum1, GCET1, FoxP1, and BCL2 have been previously published.^{12,25,26} The GCB/non-GCB subgroup status was determined using the algorithm of Choi et al for the 87 cases from UNMC.²⁵ For the LLMPP R-CHOP series and the two validation cohorts, subgroup classification was based on gene expression profiling (GEP) classifier.⁹ Double immunostaining for PY-STAT3 and CD20 was performed on a subset of the primary cohort ($n = 185$, as described above) to evaluate tumor cell-specific PY-STAT3 expression (Data Supplement). The percentage of positive cells and the intensity of PY-STAT3 staining were independently scored. A four-tiered scale (negative, 0; weak, 3; medium, 6; strong, 9) was used to grade the staining intensity in tumor cells compared with reactive T-lymphocytes, which have strong PY-STAT3 expression (score 9). A 10-tiered scale (10% to 100%) was used to score the percentage of PY-STAT3 positive tumor cells. The product of the intensity and percentage of positive cells was used as the case score with a value ≥ 15 considered positive (eg, $\geq 50\%$ positive tumor cells with an intensity of 3, or 25% positive cells with an intensity of 6).

STAT3 Knock-Down and GEP Analysis

STAT3 knock-down in DLBCL cell lines (Data Supplement), GEP data analysis, and development and validation of the 11-gene PY-STAT3 signature are described in the Data Supplement.

Table 1. Clinical Features of Patients With DLBCL According to PY-STAT3 Expression

Clinical Feature	PY-STAT3 Expression				P
	Negative (n = 116)		Positive (n = 69)		
	No.	%	No.	%	
Age, years					
Median	63		66		
Range	19.6-87.2		23.6-89.2		
< 60	58	50	27	39	.20
≥ 60	58	50	42	61	
Gender					
Male	67	58	35	49	.44
Female	49	42	34	51	
Karnofsky performance score					
> 70	98	88	58	87	1.0
≤ 70	14	12	9	13	
Ann Arbor stage					
I/II	60	54	27	42	.17
III/IV	52	46	38	58	
Extranodal sites, No.					
< 2	99	88	55	82	.42
≥ 2	14	12	12	18	
Serum lactate dehydrogenase					
Normal	71	64	33	54	.27
Elevated	40	36	28	46	
International Prognostic Index risk group					
Low (0-2)	88	80	41	67	.09
High (3-5)	22	20	20	32	
DLBCL subtypes					
GCB	67	58	29	40	.03
ABC/non-GCB	41	35	37	54	
Nonclassifiable	8	7	3	6	

NOTE. χ^2 test was used to compare the distribution of clinical features between PY-STAT3 negative and positive cases.

Abbreviations: ABC, activated B-cell-like; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like.

Fig 1. STAT3 activation is associated with poor survival in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). (A) Overall survival (OS) and (B) event-free survival (EFS) of patients with DLBCL by PY-STAT3 expression. (C) OS and (D) EFS of patients with activated B-cell/non-germinal center B-cell-like (non-GCB) DLBCL by PY-STAT3 expression. (E) OS and (F) EFS of patients with GCB-DLBCL by PY-STAT3 expression.

Statistical Analysis

Clinical and pathologic characteristics of the patients were compared by χ^2 test. The Kaplan-Meier method was used to estimate the overall survival (OS) and event-free survival (EFS) distributions, with the log-rank test performed to compare the survival curves. Univariable and multivariable Cox proportional hazards regression models were used to evaluate proposed prognostic factors. Concordance between PY-STAT3 IHC scores and the PY-STAT3 signature expression was examined by Spearman's rank correlation test. All reported *P* values are two-sided, and those less than .05 were considered statistically significant. SAS software (SAS Institute, Inc., Cary, NC) was used in all analyses.

RESULTS

PY-STAT3 Positivity Is Preferentially Associated With ABC-DLBCL

We measured tumor cell-specific PY-STAT3 activation in a subset of our primary cohort by IHC (*n* = 185). According to our scoring criteria, 69 (37%) cases were positive. The baseline clinical features were not significantly different between the PY-STAT3-positive and -negative cases (Table 1). However, the ABC/non-GCB subgroup contained significantly more PY-STAT3-positive cases than did the GCB-subgroup (47% [37 of 78] *v* 30% [29 of 96]; *P* = .03). Consistent with our previous reports on two different cohorts,^{7,8} high *STAT3* mRNA expression also occurred preferentially in the ABC subgroup (Data Supplement).

PY-STAT3 Activation Is Associated With Poor Survival in DLBCL

As expected, the IPI and COO classifiers were prognostic for OS and EFS in the entire cohort (Data Supplement). PY-STAT3-positive cases had inferior survival compared to that of negative cases in the entire cohort (*n* = 185; Figs 1A and 1B) as well as in the ABC/non-GCB subgroup (Figs 1C and 1D), but not among the GCB-DLBCL cases (Figs 1E and 1F). Multivariable analysis showed that PY-STAT3 positivity had prognostic significance for both OS and EFS independent of IPI and BCL2 expression status (Table 2). In univariable analysis, PY-STAT3 status was a significant prognosticator in the entire cohort (for both OS and EFS) as well as in the ABC/non-GCB subgroup (for EFS), but not in the GCB subgroup (Table 2). As we previously reported,²⁶ the prognostic value of BCL2 expression was restricted to the GCB subgroup. These results further support the observation based on Kaplan-Meier estimates and indicate that STAT3 activation identifies a subset of patients with DLBCL, particularly among the ABC/non-GCB subgroup, who are at high risk when treated with R-CHOP.

We also examined the utility of combining PY-STAT3 and BCL2 expression.²⁶ Interestingly, BCL2 and PY-STAT3 double negative patients showed an exceedingly favorable prognosis (OS, *P* < .001; EFS, *P* < .001, estimated 5-year and 10-year OS of 91%) compared with those positive for either one or both markers (Data Supplement). We

Table 2. Multivariable and Univariable Analyses by Cox Proportional Hazard Model

Survival	Covariates	HR	95% CI	<i>P</i>
Multivariable analysis for IPI, PY-STAT3, and BCL2 as covariates in the entire cohort (<i>n</i> = 309)				
OS	IPI (high: 3-5 <i>v</i> low: 0-2)	2.3	1.1 to 4.7	.02
	PY-STAT3 (pos. <i>v</i> neg.)	2.3	1.1 to 4.6	.02
	BCL2 (pos. <i>v</i> neg.)	2.1	1.01 to 4.5	.046
EFS	IPI (high: 3-5 <i>v</i> low: 0-2)	1.9	0.97 to 3.7	.06
	PY-STAT3 (pos. <i>v</i> neg.)	2.2	1.1 to 4.1	.02
	BCL2 (pos. <i>v</i> neg.)	1.8	0.94 to 3.5	.08
Univariable analysis for each covariate in the entire cohort (<i>n</i> = 309)				
OS	Subtype (ABC <i>v</i> GCB)	2.1	1.4 to 3.2	< .01
	IPI (high: 3-5 <i>v</i> low: 0-2)	2.7	1.8 to 4.2	< .01
	PY-STAT3 (pos. <i>v</i> neg.)	1.9	1.2 to 3.2	.01
	BCL2 (pos. <i>v</i> neg.)	2.1	1.3 to 3.6	< .01
EFS	Subtype (ABC <i>v</i> GCB)	2.3	1.6 to 3.3	< .01
	IPI (high: 3-5 <i>v</i> low: 0-2)	2.5	1.7 to 3.6	< .01
	PY-STAT3 (pos. <i>v</i> neg.)	1.9	1.2 to 2.9	< .01
	BCL2 (pos. <i>v</i> neg.)	2.1	1.3 to 3.4	< .01
Univariable analysis for each covariate in the GCB subgroup (<i>n</i> = 153)				
OS	IPI (high: 3-5 <i>v</i> low: 0-2)	3.4	1.7 to 6.8	< .01
	PY-STAT3 (pos. <i>v</i> neg.)	1.6	0.8 to 3.5	.2
	BCL2 (pos. <i>v</i> neg.)	2.9	1.01 to 8.4	.047
EFS	IPI (high: 3-5 <i>v</i> low: 0-2)	3.2	1.7 to 5.9	< .01
	PY-STAT3 (pos. <i>v</i> neg.)	1.6	0.8 to 3.2	.18
	BCL2 (pos. <i>v</i> neg.)	4.8	1.8 to 12.8	< .01
Univariable analysis for each covariate in the ABC/non-GCB subgroup (<i>n</i> = 125)				
OS	IPI (high: 3-5 <i>v</i> low: 0-2)	1.9	1.1 to 3.2	.02
	PY-STAT3 (pos. <i>v</i> neg.)	1.9	0.95 to 3.9	.07
	BCL2 (pos. <i>v</i> neg.)	1.5	0.75 to 2.9	.25
EFS	IPI (high: 3-5 <i>v</i> low: 0-2)	1.7	1.0 to 2.7	.04
	PY-STAT3 (pos. <i>v</i> neg.)	2	1.07 to 3.8	.03
	BCL2 (pos. <i>v</i> neg.)	1.1	0.63 to 2.1	.66

Abbreviations: ABC, activated B-cell-like; EFS, event-free survival; GCB, germinal center B-cell-like; HR, hazard ratio; IPI, International Prognostic Index; OS, overall survival.

Fig 2. High *STAT3* mRNA expression is associated with poor survival in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). (A) Overall survival and (B) event-free survival of patients with DLBCL by the expression levels of *STAT3* mRNA. Int, intermediate.

obtained similar findings when combining *BCL2* and *STAT3* mRNA expression. Furthermore, the combined PY-*STAT3* and *BCL2* status was associated with survival outcome only in the GCB- but not the ABC-DLBCL subgroups (Data Supplement). This subgroup specificity likely reflects a dominant prognostic impact by *BCL2* in the GCB-DLBCL subgroup (Table 2).²⁶

High *STAT3* mRNA Is Associated With Poor Survival in DLBCL

We also examined the prognostic value of *STAT3* mRNA expression in the LLMPP R-CHOP series ($n = 222$). Using the average intensity of the three *STAT3* probesets, we divided the cases into three subgroups: low ($< \text{mean} - \text{one standard deviation [SD]}$, $n = 37$), high ($> \text{mean} + \text{one SD}$, $n = 29$), and intermediate (the remaining cases, $n = 156$). Clinical characteristics of the patients in these three groups were not significantly different; however, the subgroup with the highest levels of *STAT3* mRNA was enriched in ABC-DLBCL subtype and cases stained positive for PY-*STAT3* (Data Supplement), likely reflecting the fact that *STAT3* is a positively auto-regulated gene.²⁷ Similar to our observations on PY-*STAT3*, cases with high *STAT3* mRNA had significantly worse prognosis (OS, $P = .004$; EFS, $P = .003$; Fig 2). However, *STAT3* mRNA was not a significant prognosticator when the cohort was divided into COO subgroups.

Development of a GEP-based PY-*STAT3* Signature for DLBCL Prognostication

To further evaluate the prognostic significance of PY-*STAT3*, we constructed a GEP-based PY-*STAT3* signature using differential gene expression on *STAT3* knockdown in ABC-DLBCL cell lines, presence of at least one *STAT3* binding site in the promoter region of putative target genes, and differential expression between PY-*STAT3*-positive and -negative DLBCL tumors (t -test $P < .05$; fold change > 2). This analysis leads to a set of 265 candidate genes (347 probe sets) that correlated with *STAT3* activation status in DLBCL. Known *STAT3* target genes such as *CD48*, *CD96*, *IRF1*, *IL10*, *BCL3*, and *IL2RB*

were highly expressed in the PY-*STAT3*-positive tumors,²⁸⁻³⁰ whereas the PY-*STAT3*-negative tumors expressed high levels of *RAC1*, *MAPK1* and *AKT2* (Fig 3).

Next, to enrich for genes with greatest prognostic values, we used a semi-supervised prediction algorithm.^{31,32} The OS response from the LLMPP R-CHOP cohort ($n = 222$) was used at this step. Using a significance level $\leq .05$, we obtained a list of 36 genes and subsequently filtered them for concordance expression in patient and cell line GEP data (Data Supplement). This five-step procedure finally produced an 11-gene PY-*STAT3* signature (Fig 3). In the subsequent prognostic analyses, the averaged expression level of the 11 member genes is used as the signature score to correlate with DLBCL survival risk.

Three types of tests were performed to confirm that this GEP signature can reliably report PY-*STAT3* activity. First, we validated four of the 11 genes by quantitative reverse transcriptase polymerase chain reaction in two ABC-DLBCL cell lines treated with *STAT3* siRNA (Data Supplement). Second, applying Spearman's correlation test to the 98 cases for which both the IHC score and GEP data were available (Data Supplement), we established that the 11-gene signature strongly correlated with the IHC-based PY-*STAT3* status in the entire cohort (coefficient $r = 0.47$; $P < .001$) as well as in the ABC ($r = 0.55$; $P < .001$) and GCB ($r = 0.47$; $P = .001$) subgroups. Third, after dividing three DLBCL cohorts (including primary cohort and two independent validation cohorts) into quartile subgroups using the signature expression, we demonstrate that ABC-DLBCL cases are significantly enriched in the intermediate/high and high quartile groups in all three cohorts (Data Supplement).

Eleven-Gene PY-*STAT3* Signature Is Associated With Poor Survival in R-CHOP-Treated DLBCL

First, we compared the survival response of the quartile subgroups among the LLMPP R-CHOP cohort. As shown in Figures 4A and 4B, we detected significant survival differences among these quartile subgroups with 5-year OS rates at 84%, 81%, 57%, and 48%, and 5-year EFS rates at 81%, 77%, 51%, and 40% for quartiles 1, 2, 3, and

Fig 3. Development of a PY-STAT3-based gene signature to predict the survival of patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Detailed description of the procedure is provided in the Data Supplement. The heat map shows the differential expression pattern of *STAT3* candidate genes between PY-STAT3-positive and -negative cases. PY-STAT3 immunohistochemistry (IHC) scores and *STAT3* mRNA levels are illustrated in the top of the heat map. Green and red colors in the heat map indicate relatively low and high gene expression, respectively, as indicated. GEP, gene expression profiling; SSP, semi-supervised prediction.

4, respectively. Among the ABC-DLBCL cases, those in the first quartile (lowest expression) had a more favorable outcome than did those in the other three quartiles (Figs 4C and 4D). In comparison, such quartile-associated survival differences were not detected among the GCB-DLBCL cases (not shown).

Since the LLMPP R-CHOP cohort was also used to develop the PY-STAT3 signature, it is important to confirm the validity of this 11-gene model using independent cohorts. In the absence of another large-scale R-CHOP cohort that features complete and publically accessible GEP and treatment response data, we turned to the LLMPP CHOP cohort¹³ and a small R-CHOP series²⁴ for validation analyses (Data Supplement). The clinical features of these two cohorts are comparable with our primary cohort with the exception of serum lactate dehydrogenase, which was elevated more often in the CHOP series than in our primary R-CHOP cohort (Data Supplement). We used two validation approaches. First, survival analysis showed that

high expression of the 11-gene signature was associated with poor OS in the LLMPP CHOP cohort (Data Supplement; $n = 181$). A similar trend was observed in the small R-CHOP series; however, the prognostic difference did not reach statistical significance, likely because of the small cohort size (Data Supplement; $n = 65$). Second, we conducted a calibration analysis to directly compare model-predicted and observed survival probability.³³ As shown in the Data Supplement, this analysis revealed good agreement at multiple time points between prediction by the 11-gene signature and observed OS risk in the intermediate and low-risk groups in the small R-CHOP cohort and in the high-risk group in the LLMPP CHOP cohort. Given the known survival differences between CHOP and R-CHOP regimens, results from this validation analysis suggest to us that the prognostic value of the 11-gene PY-STAT3 signature is likely applicable to the general setting of DLBCL treated with CHOP-containing therapies.

Fig 4. The 11-gene PY-STAT3 signature was predictive of survival of patients with diffuse large B-cell lymphoma (DLBCL) in the entire cohort (A, B) and in the activated B-cell DLBCL subgroup (C, D). Overall and event-free survival are shown using the expression quartiles (Q) of the 11-gene signature.

DISCUSSION

In this retrospective analysis, we demonstrated that PY-STAT3 activation is prognostic for poor survival in patients with DLBCL treated with R-CHOP, the current standard of care for DLBCL. The prognostic significance of PY-STAT3 is independent of International Prognostic Index and BCL2 expression, and particularly strong among the ABC-DLBCL subgroup. Our results also demonstrated the prognostic value of combining PY-STAT3/BCL2 expression for all patients with DLBCL and GCB-DLBCL. Furthermore, we report an 11-gene signature that can be used to track STAT3 activation status and correlate with poor outcome of patients with DLBCL treated with either CHOP or R-CHOP. To the best of our knowledge, this represents the largest and most comprehensive study to date demonstrating the prognostic significance of STAT3 activation in patients with DLBCL.

STAT3 activation determined by IHC is reportedly associated with poor prognosis in many cancers³⁴⁻³⁶; however, a recent study failed to identify prognostic value for PY-STAT3 in DLBCL.³⁷ Compared with that report, many more R-CHOP cases were examined in this study (185 v 38). More importantly, we used CD20/PY-STAT3 double staining to determine tumor-specific STAT3 activation, thus avoiding interference from stromal cells and reactive T-lymphocytes.¹³ Admittedly, although we have validated the 11-gene PY-STAT3 signature using two independent cohorts, it is important to further test and extend its prognostic utility in prospective cohorts. Tracking activated STAT3 by a GEP-based signature is relatively new and has not been appreciated in clinical oncology. Yet, a GEP-based prognosticator, once fully validated, can be further developed using emerging technologies such as diagnostic gene chips at the point of care.

Before this report, two GEP-based DLBCL prognostic models have been reported by the LLMP consortium, namely the bivariate

GCB/ABC model^{4,5} and the trivariate model derived from the GCB/ABC, stromal-1, and stromal-2 GEP signatures.¹³ Interestingly, although our 11-gene signature is a much simpler univariate predictor, its predictive power is quite comparable to the trivariate model. One possible explanation comes from the fact that STAT3 activation within the tumor cells is a comprehensive readout of both the intrinsic genetic alterations and the tumor-microenvironment cross-talk. It is worth noting that GEP-based PY-STAT3 positivity reported here is much more common than any single reported driver mutation in DLBCL, implying that STAT3 activation is a shared downstream oncogenic event that can be triggered by different genetic alterations. In addition, the genes that define the ABC-DLBCL signature were expressed at equivalent levels in subgroups of different PY-STAT3 status, suggesting that STAT3 activation is regulated independently from the defining features of the ABC-DLBCL subgroup.

The PY-STAT3 signature reported here may provide useful insights into the role of STAT3 in oncogenesis and possibly also therapeutic response in DLBCL. Six of the 11 genes in the signature have been functionally studied and five of them have been implicated in cancer. HSD17B4 is a dehydrogenase involved in peroxisomal fatty acid beta-oxidation, and its overexpression was prognostic in prostate cancer.³⁸ SLC2A13 encodes a H⁺-myo-inositol transporter that serves as a marker for cancer stem cells in oral squamous cell carcinoma.³⁹ Aberrant expression of MT1X has been observed in several kinds of carcinomas and was correlated with poor therapeutic response.^{40,41} RHEB is a key regulator in the PI3K/Akt/mTOR pathway, which directly activates mTORC1 activity.⁴² Cell type-specific oncogenic activity has been shown for RHEB, especially in the context of PTEN haploinsufficiency.⁴³ This is particularly interesting in light of our previous report that PTEN loss occurs in 11% of GCB-DLBCL.⁴⁴ Finally, ZNF420 encodes the KRAB-type zinc finger protein Apak, which has been implicated in DNA damage response.⁴⁵

Our finding in this report also has therapeutic implications regarding the potential utility of targeting STAT3 directly. We and others have previously reported that blocking JAK/STAT3 activation in ABC-DLBCL cell lines can significantly reduce cell proliferation and survival.^{8,22} Our results, when combined with these previous observations, further strengthen the rationale of targeting STAT3 directly for treatment of DLBCL, especially the ABC-DLBCL subtype.

In summary, we have demonstrated in this study that STAT3 activation status either using on an IHC method or an 11-gene GEP signature is correlated with poor prognosis of patients with DLBCL treated with the R-CHOP regimen. Our findings support the hypothesis that targeting the STAT3 signaling pathway, either with

monotherapy or in combination with R-CHOP, may improve the survival of patients with DLBCL carrying PY-STAT3 positive tumors. Finally, none of the 11 genes in the PY-STAT3 signature have been previously shown to be a transcriptional target of STAT3 in DLBCL, and their potential contributions to lymphoma pathobiology await future investigation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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