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LETTER TO EDITOR

BCL-2 overexpression overcomes cell of origin stratification in diffuse large B-cell lymphoma

Moussab Damlaj [a,b,c,*], Rehab Yassin [a], Saeed Al-Shieban [d], Tabreez Pasha [a], Mohsen Alzahrani [a,b,c], Ayman Alhejazi [a,b,c], Ahmad Alaskar [a,b,c]

[a] Division of Hematology & HSCT, Department of Oncology, King Abdulaziz Medical City, Riyadh, Saudi Arabia
[b] King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
[c] King Abdullah International Medical Research Center, Riyadh, Saudi Arabia
[d] Department of Pathology & Laboratory Medicine, Riyadh, Saudi Arabia

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To the Editor

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with variable clinical and pathologic presentations. The prognosis of DLBCL patients can be estimated using the International Prognostic Index (IPI) or its revised variant (R-IPI) [1]. Furthermore, using gene expression profiling, DLBCL can be divided based on its cell of origin (COO) into germinal center type (GCB), activated B-cell, or type 3 with the latter two collectively known as non-germinal center type (non-GCB) with poor prognosis relative to the former type [2]. However, such complementary DNA (cDNA)-based assays needed for classification remain prohibitive for routine clinical use almost two decades after the initial report. Immunohistochemical (IHC) algorithms, such as the one proposed by Hans et al., [3] are rapid alternatives that are readily available and have demonstrated reasonable concordance to gene expression profiling.

The antiapoptotic B-cell lymphoma 2 (BCL-2) oncogene confers a negative prognostic impact in GCB DLBCL when overexpressed irrespective of MYC status [4]. Non-GCB DLBCL appears to arise from postgerminal center B cells and involves activation of the nuclear factor signaling pathway (NFκB). BCL-2 overexpression can also be seen in conjunction with MYC, resulting in the entity called ''double expressing lymphoma'' [5]. Our aim with this analysis was twofold: first, to assess the prognostic impact of COO assignment using the Hans algorithm in DLBCL patients from the Middle East and North Africa (MENA) region; and second, to examine whether BCL-2 overexpression alters the expected COO-based prognosis.

After due Institutional Review Board approval, adult patients with newly diagnosed DLBCL at our center between 2010 and 2015 were identified. Clinical and pathologic variables were retrospectively abstracted. COO analysis was determined by the Hans criteria as previously described [3]. Categorical and continuous variables were compared using chi-square and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log ranks. Relapse, progression, or

* Corresponding author at: Division of Hematology & HSCT, Department of Oncology, King Abdul-Aziz Medical City, P.O. Box 22490, Riyadh 11426, Saudi Arabia.
E-mail address: damlajmo@ngha.med.sa (M. Damlaj).

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death was considered an event for progression-free survival (PFS) estimation. Analysis was computed using the JMP Pro Version 11 (SAS Institute, Cary, NC, USA) software and EZR on R Commander Version 1.35 [6].

A total of 122 patients were identified and analyzed. Median follow-up of the entire cohort was 32.9 (0.2—123.7) months, during which the estimated 3-year overall survival (OS) was 66.1% and PFS was 55.1%. Stratifying patients to GCB and non-GCB, baseline characteristics between the strata with regard to age, sex, stage, performance status, lactate dehydrogenase level, extranodal disease, BCL-2 expression, IPI, therapy delivered, and the use of involved field radiotherapy were similar (Table 1). A majority of patients were treated with combinational chemotherapy containing rituximab and 13 patients died without receiving any therapy. The estimated PFS at 3 years was significantly higher for GCB versus non-GCB at 66.9% versus 43.9%, respectively ($p = .016$), but OS was similar at 71.9% versus 62.4% ($p = .24$; Fig. 1). However, stratifying patients by COO and positive BCL-2 expression showed that the estimated 3-year OS was similar at 58.7% versus 52.8% for GCB and non-GCB, respectively. Similarly, patients with negative BCL-2 expression had a superior estimated 3-year OS at 77.3% versus 75.4% for GCB and non-GCB ($p = .035$), respectively, as shown in Fig. 2. Thus, the negative prognosis portended by COO assignment was overcome by the impact of BCL-2 expression.

Since the original depiction of COO assignment using cDNA microarrays, significant effort has been made to establish surrogate techniques that can be widely adopted for routine practice. More than one IHC criteria were described and the Hans algorithm is among the most widely used due to its relative ease of application. However, concordance with gene expression is not absolute and other

Table 1 Baseline characteristics of patients stratified by cell of origin.

Characteristic	GCB ($n = 59$)	Non-GCB ($n = 63$)	p
Age, median (range)	60 (18—95)	65 (21—98)	.25
Gender, *n* (%)			.65
Male	37 (63)	37 (59)	
Female	22 (37)	26 (41)	
Stage, *n* (%)			.62
I/II	11 (19)	14 (22)	
III/IV	48 (81)	49 (78)	
ECOG, *n* (%)			.24
0—2	37 (64)	30 (49)	
3—4	18 (31)	28 (46)	
N/A	3 (5)	3 (5)	
Lactate dehydrogenase, *n* (%)			.68
Normal	13 (22)	11 (18)	
High	42 (73)	49 (79)	
N/A	3 (5)	2 (3)	
Extranodal disease, *n* (%)	21 (36)	20 (33)	.69
BCL-2 expression, *n* (%)	26 (44)	31 (54)	.51
IPI, *n* (%)			.26
Low	19 (32)	12 (19)	
Low to intermediate	11 (19)	9 (14)	
High to intermediate	13 (22)	19 (30)	
High	16 (27)	23 (37)	
Chemotherapy used, *n* (%)			.88
R-CHOP	43 (73)	45 (71)	
R-CHOP/R-CVP	8 (14)	10 (16)	
R-CVP	1 (2)	2 (3)	
Palliative	2 (3)	3 (5)	
Not treated	5 (8)	3 (5)	
Involved field radiotherapy, *n* (%)	16 (27)	17 (27)	.99
End of treatment response, *n* (%)			.66
Complete response	40 (68)	36 (57)	
Partial response	5 (8)	8 (13)	
Progressive disease	6 (10)	9 (14)	
N/A	8 (14)	10 (16)	
Autologous HSCT, *n* (%)	2/8 (25)	9/20 (45)	.32
Follow-up months, median (range)	35 (0.2—123.7)	29.9 (0.27—100.3)	.45

BCL-2 = B-cell lymphoma 2; CHOP = rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone; CVP = rituximab with cyclophosphamide, vincristine, and prednisone; ECOG = Eastern Cooperative Oncology Group; GCB = germinal center B-cell lymphoma; HSCT = hematopoietic stem cell transplantation; IFRT = involved field radiotherapy; IPI = International Performance Index; N/A = not available.

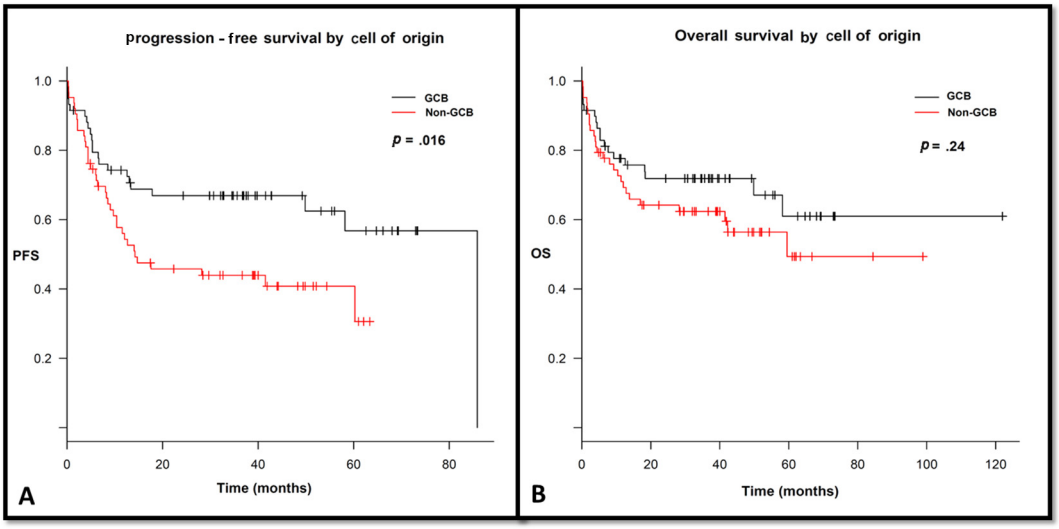

Fig. 1 Progression free survival (PFS) and overall survival (OS) by cell of origin (COO). GCB = germinal center B-cell lymphoma.

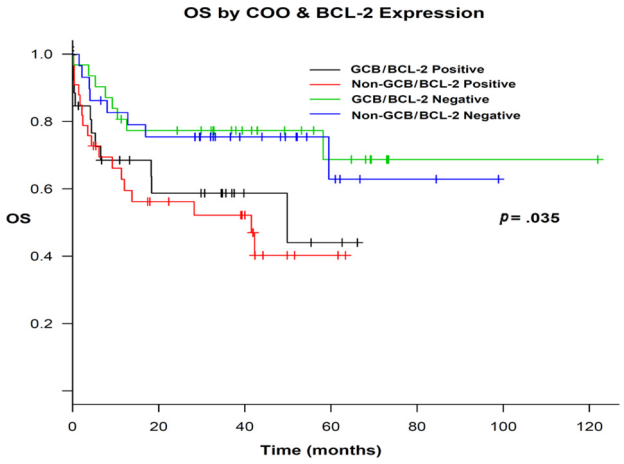

Fig. 2 Overall survival (OS) by cell of origin (COO) and B-cell lymphoma 2 (BCL-2) expression. GCB = germinal center B-cell lymphoma.

efforts of classification using formalin-fixed paraffin-embedded tissue such as the Lymph2Cx emerged as a promising test but remains to be further validated and commercialized [7,8].

Recent attempts have been made toward improving the worse prognosis observed in non-GCB DLBCL. In a Phase II trial, combining lenalidomide with the backbone of rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP) improved outcomes compared with historical controls, and a Phase III trial (ROBUST) is currently ongoing to confirm these observations [9]. Previously, the addition of the proteasome inhibitor bortezomib did not improve PFS in non-GCB DLBCL [10]. Despite such disappointment, the quests to improve results seen with R-CHOP remain ongoing. The recent approval of the BCL-2 inhibitor venetoclax (previously ABT-199) for the treatment of chronic lymphocytic leukemia (CLL) expanded the spectrum of targeted therapy in oncology [11]. Current efforts are underway to examine its efficacy in other malignancies. Mutations within BCL-2, especially those arising from t(14;18)(q32;q21), are typically detected in the GCB subtype of DLBCL, but increased protein expression can also be due to post-transcriptional mechanisms notwithstanding translocations [12].

Herein, we demonstrated that COO assignment resulted in improved PFS but not OS in GCB versus non-GCB patients from the MENA region. It is possible that a larger sample size could have detected a survival difference given the divergent OS curves observed. That said, such COO-based prognosis was nullified when stratified by BCL-2 overexpression. Importantly, however, BCL-2 expression quantification via IHC requires standardization for optimal scoring and some preliminary work has emerged with this regard recently [13]. Our analysis is limited by its retrospective nature and sample size. Furthermore, IHC staining for the MYC oncogene was not performed resulting in our inability to identify ''double expresser'' lymphoma. However, the inferior outcome in cases of MYC overexpression is only observed if BCL-2 is co-expressed [5]. Despite these limitations, this report generates additional insight that IHC methodology can discriminate outcome in patients from the MENA region using tools that are readily available in all pathology labs and would have implications for routine clinical practice. In conclusion, our results show that COO assignment using IHC demonstrated superior PFS for GCB over non-GCB; however, this was mitigated by BCL-2 overexpression. This raises possibilities regarding further exploration of the currently available BCL-2 inhibitors. These observations warrant further study.

Conflicts of interest

There are no relevant conflicts of interests for any of the authors.

References

[1] Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients

with diffuse large B-cell lymphoma treated with R-CHOP. Blood 2007;109:1857—61.
[2] Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503—11.
[3] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275—82.
[4] Visco C, Tzankov A, Xu-Monette ZY, Miranda RN, Tai YC, Li Y, et al. Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: a report from an International DLBCL rituximab-CHOP Consortium Program Study. Haematologica 2013;98:255—63.
[5] Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3452—9.
[6] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452—8.
[7] Yoon N, Ahn S, Yoo HY, Kim SJ, Kim WS, Ko YH. Cell-of-origin of diffuse large B-cell lymphomas determined by the Lymph2Cx assay: better prognostic indicator than Hans algorithm. Oncotarget 2017;8:22014—22.
[8] Scott DW, Mottok A, Ennishi D, Wright GW, Farinha P, Ben-Neriah S, et al. Prognostic significance of diffuse large b-cell lymphoma cell of origin determined by digital gene expression in formalin-fixed paraffin-embedded tissue biopsies. J Clin Oncol 2015;33:2848—56.
[9] Nowakowski GS, Chiappella A, Witzig TE, Spina M, Gascoyne RD, Zhang L, et al. ROBUST: lenalidomide-R-CHOP versus placebo-R-CHOP in previously untreated ABC-type diffuse large B-cell lymphoma. Future Oncol 2016;12:1553—63.
[10] Leonard JP, Kolibaba KS, Reeves JA, Tulpule A, Flinn IW, Kolevska T, et al. Randomized phase II study of R-CHOP with or without bortezomib in previously untreated patients with non-germinal center B-cell-like diffuse large B-cell lymphoma. J Clin Oncol 2017;35:3538—46.
[11] Gentile M, Petrungaro A, Uccello G, Vigna E, Recchia AG, Caruso N, et al. Venetoclax for the treatment of chronic lymphocytic leukemia. Expert Opin Investig Drugs 2017;26:1307—16.
[12] Delbridge AR, Grabow S, Strasser A, Vaux DL. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. Nat Rev Cancer 2016;16:99—109.
[13] Szafer-Glusman F, Lei G, Mottok A, Farinha P, Ray J, Farazi TA, et al. BCL2 expression identifies a population with unmet medical need in previously untreated patients with DLBCL. Blood 2017;130:418.