The proportion of activated B-cell like subtype among *de novo* diffuse large B-cell lymphoma increases with age

The prognosis for elderly patients with diffuse large B-cell lymphomas (DLBCL) remains particularly poor. The most common explanation involves co-morbidities related to advanced age, which strongly impact chemotherapy feasibility and tolerance.[1] Despite a generally poor prognosis, a recent clinical trial dedicated to patients over 80 years of age demonstrated that a significant proportion of DLBCL patients could be cured using rituximab (R) and reduced-intensity chemotherapy (R-miniCHOP).[2] In addition to well-known clinical conditions related to aging, the poor prognosis of DLBCL in elderly patients may also be related to intrinsic biological features of the tumor. The germinal center B-cell like (GCB)/activated B-cell like (ABC) signature is considered a major biological determinant of prognosis, independent of the international prognostic index (IPI), remaining predictive of outcome in patients treated by immunochemotherapy.[3] However, the relationship between aging and the distribution of these two main gene expression profiles (GEP) has not been specifically studied, even though a trend for a higher proportion of ABC patients was reported by Rosenwald and colleagues in patients over 60 years of age.[4] To address this question, we retrospectively determined the GEP of a series of 131 primary *de novo* DLBCL patients over 50 years of age (median age 68 years, range 50-91 years), selected on the basis of histopathological diagnosis and available tumor RNA. The GCB/ABC signature was determined by DASL technology with RNA extracted from fresh frozen material as previously reported.[5,6] By this reproducible and robust method, we observed a concordance rate of 70-95% with immunohistochemistry.[5,6] A direct comparison with microarray-based technology using RNA extracted from formalin-fixed paraffin-embedded tissues is ongoing.

Using this approach, 51 cases (39%) were classified in the GCB group, 57 cases (44%) in the ABC group and 23 (17%) in an unclassified (intermediate) group (Figure 1A). To determine the distribution of the GCB/ABC phenotype according to age, the overall population was

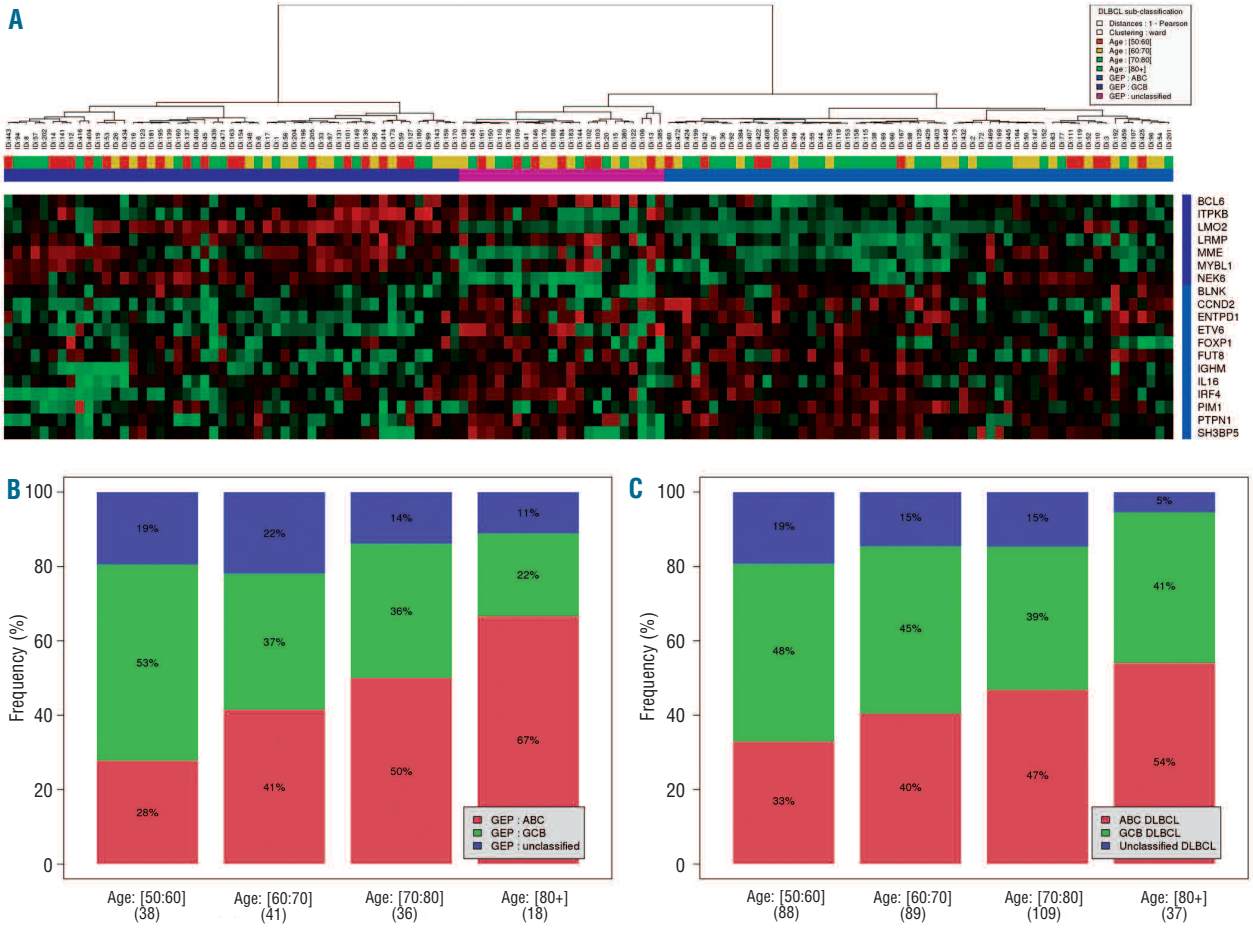

Figure 1. GCB/ABC distribution with aging in DLBCL patients older than 50 years. (A) Hierarchical clustering. DLBCL were clustered according to gene expression as assessed by DASL. Genes fitting the ABC signature and genes fitting the GCB signature are indicated on the right. Patients are identified by their unique patient numbers. A color code related to age category is indicated (upper part of the HeatMap). Unsupervised hierarchical clustering was performed using 'COR' distances (the opposite of Pearson's r) after Cubic Spline normalization (GenomeStudio V2009.1 software, Illumina). DLBCL diagnosis was performed according to WHO criteria. Primary mediastinal B-cell lymphomas and T-cell rich B-cell lymphomas were excluded. (B) ABC and non-ABC proportions according to age categories in a monocentric series of DLBCL. Number of patients by age category are indicated in brackets. (C) ABC and non-ABC proportions according to age in an independent series of DLBCL. Number of patients by age category are indicated in brackets.

divided into four age-related categories, i.e. Group 1: 50-60 years (n=36); Group 2: 60-70 years (n=41); Group 3: 70-80 years (n=36), and Group 4: 80 years and over (n=18). Figure 1B shows the rates of ABC/GCB/unclassified DLBCL according to these categories with a significant increase in the ABC subtype in proportion to increasing age. There was a significant difference in ABC/non-ABC (including GCB and unclassified) distribution between Group 1 and Group 4 (28% ABC *vs.* 67%, *P*=0.01, Fisher's exact test). To consolidate our results, we next determined whether the same aging effect was also observed in an independent published series of DLBCL (data accessible from the GEO databank, NCBI, GSE10846).[3] Despite technological differences in the determination of GEP (Affymetrix array *vs.* DASL), a similar increase in the ABC DLBCL proportion with age was observed (Figure 1C). In this series of patients over 50 years of age (median 68 years, range 50-92 years), the percentage of ABC DLBCL increases with age, with a differential distribution between patients aged between 50-60 years and those over 80 years of age (33% ABC *vs.* 54%, *P*=0.04, Fisher's exact test, Figure 1C).

We observed an average increase of 13.7% in ABC DLBCL for each ten years of aging after the age of 50 years in our series and of 7.5% in the Lenz series. A relationship between GCB/ABC distribution and age has been suggested in the context of pediatric lymphoma. Immunohistochemistry analysis indicated that, in this setting, DLBCL is associated most strongly with the GCB subgroup, which reflects the opposite pattern to that observed in the elderly.[7] Whether a continuous increase in the ABC DLBCL in proportion to age can be observed remains to be determined.

Attempts to explain such a skewed ABC distribution during aging remain speculative. Diversity of normal repertoire of B/plasma cells is reduced with age, and this loss of diversity is characterized by clonal expansions of B cells *in vivo*.[8] For instance, an increase in the number of B cells expressing the *VH4-34 IgH* gene has been noted during aging.[9] We have previously shown that *VH4-34* + DLBCL frequently expressed IgM, frequently displayed the t(3;14)(q27;q32) translocation, and are typically classified in the ABC subtype.[5,10] This suggests that the increase in the proportion of ABC DLBLC with age may reflect a change in the B-cell population during aging. Another hypothesis relates to the putative pathological specificity of DLBCL occurring in elderly patients. EBV-related DLBCL are almost exclusively reported in elderly or very old patients, but this provisional WHO entity seems mostly limited to Asia and is rare in Western countries.[11]

Age is an obvious and major prognostic factor which impacts directly on treatment strategies, chemotherapy feasibility and tolerance. This precludes, therefore, any comparison of survival between young and elderly patients with ABC DLBCL. To confirm the prognostic value of the ABC/GCB signature regardless of age, we

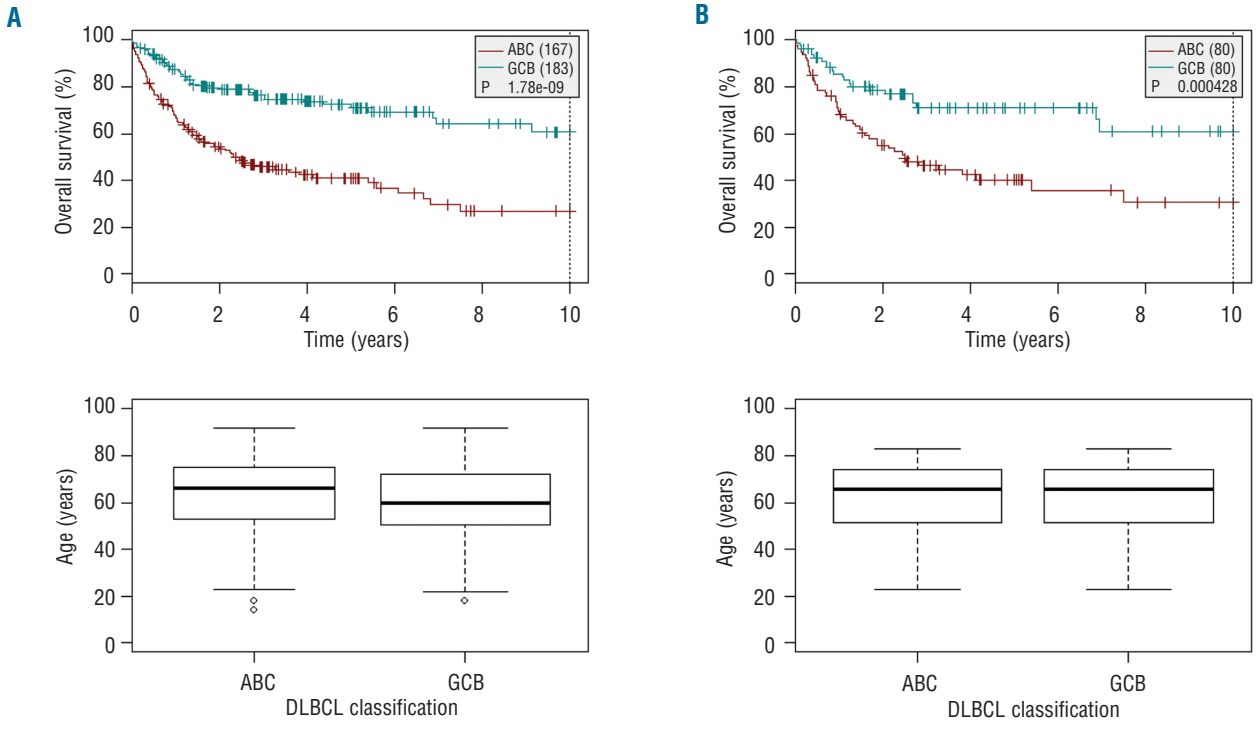

Figure 2. Impact of the GCB/ABC molecular classification in age-matched patients. Random combinations of patients were produced from the series published by Lenzet *et al.* (n=350). For each patient randomly selected in the GCB group, a patient with the same age (in years) was randomly selected in the ABC group, in order to form 80GCB/ABC pairs of age-matched patients. In the 50 subseries produced, 91% of the 350 samples were selected at least once, and all Log rank *P* values were significative (max=0.002). (A) Overall survival in the overall population. (B) Example of survival curves obtained in a series of 80 age-matched patients.

compared overall survival according to molecular classification by producing random pairs of age-matched patients from the publicly available data published by Lenz *et al.*[12] For each patient randomly selected in the GCB subtype, an age-matched patient was randomly selected from the ABC subtype, in order to form 80 GCB-ABC pairs of patients. In the 50 tested random paired combinations, the ABC subtype remains constantly correlated to an unfavorable outcome indicating that the unfavorable prognostic value of the molecular signature is not related to the skewed ABC distribution during aging (Figure 2).

In conclusion, our results indicate that in addition to constitutive factors related to advanced age, the prognosis of DLBCL is also conditioned by intrinsic biological features of the tumor cells. Despite promising results obtained using conventional immuno-chemotherapy, such as R-miniCHOP, new therapeutic strategies in geriatric populations should include molecules able to target oncogenic pathways related to the ABC phenotype, such as the NFKB pathway.

Sylvain Mareschal,[1,2] Hélène Lanic,[1,3] Philippe Ruminy,[1] Christian Bastard,[1] Hervé Tilly,[1,3] and Fabrice Jardin[1,3]

[1]UMR INSERM U918, Centre Henri Becquerel, University of Rouen, Rouen; [2]University of Rouen, MSc Bioinformatics, Mont Saint-Aignan; and [3]Department of Haematology, Centre Henri Becquerel, Rouen, France

Correspondence: Fabrice Jardin, UMR INSERM U918, Centre Henri Becquerel, University of Rouen, Rouen, France.
E-mail: fabrice.jardin@rouen.fnclcc.fr

Key words: gene expression profile, diffuse large B-cell lymphoma, aging.

Citation: Mareschal S, Lanic H, Ruminy P, Bastard C, Tilly H, Jardin F. The proportion of activated B-cell like subtype among de novo diffuse large B-cell lymphoma increases with age. Haematologica 2011;96(12):1888-1890 doi:10.3324/haematol.2011.050617

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Kobayashi Y, Miura K, Hojo A, Hatta Y, Tanaka T, Kurita D, et al. Charlson Comorbidity Index is an independent prognostic factor among elderly patients with diffuse large B-cell lymphoma. J Cancer Res Clin Oncol. 2011;137(7):1079-84.
2. Peyrade F, Jardin F, Thieblemont C, Thyss A, Emile JF, Castaigne S, et al. Attenuated immunochemotherapy regimen (R-miniCHOP) in elderly patients older than 80 years with diffuse large B-cell lymphoma: a multicentre, single-arm, phase 2 trial. Lancet Oncol. 2011;12(5):460-8.
3. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359(22):2313-23.
4. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(25):1937-47.
5. Ruminy P, Etancelin P, Couronné L, Parmentier F, Rainville V, Mareschal S, et al. The isotype of the BCR as a surrogate for the GCB and ABC molecular subtypes in diffuse large B-cell lymphoma. Leukemia. 2011;25(4):681-8.
6. Lanic H, Mareschal S, Mechken F, Picquenot JM, Cornic M, Maingonnat C, et al. Interim positron emission tomography scan associated with international prognostic index and germinal center B cell-like signature as prognostic index in diffuse large B-cell lymphoma. Leuk Lymphoma. 2011 Aug 1. [Epub ahead of print]
7. Oschlies I, Klapper W, Zimmermann M, Krams M, Wacker HH, Burkhardt B, et al. Diffuse large B-cell lymphoma in pediatric patients belongs predominantly to the germinal-center type B-cell lymphomas: a clinicopathologic analysis of cases included in the German BFM (Berlin-Frankfurt-Munster) Multicenter Trial. Blood. 2006;107(10):4047-52.
8. Gibson KL, Wu YC, Barnett Y, Duggan O, Vaughan R, Kondeatis E, et al. B-cell diversity decreases in old age and is correlated with poor health status. Aging Cell. 2009;8(1):18-25.
9. Wang X, Stollar BD. Immunoglobulin VH gene expression in human aging. Clin Immunol. 1999;93(2):132-42.
10. Jardin F, Sahota SS, Ruminy P, Parmentier F, Picquenot JM, Rainville V, et al. Novel Ig V gene features of t(14;18) and t(3;14) de novo diffuse large B-cell lymphoma displaying germinal center-B cell like and non-germinal center-B cell like markers. Leukemia. 2006;20(11):2070-4.
11. Hofscheier A, Ponciano A, Bonzheim I, Adam P, Lome-Maldonado C, Vela T, et al. Geographic variation in the prevalence of Epstein-Barr virus-positive diffuse large B-cell lymphoma of the elderly: a comparative analysis of a Mexican and a German population. Mod Pathol. 2011;24(8):1046-54.
12. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, Davis RE, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA. 2008; 105(36):13520-5.

Bortezomib and high-dose melphalan conditioning for stem cell transplantation for AL amyloidosis: a pilot study

Treatment with high-dose intravenous melphalan followed by autologous stem cell transplantation (HDM/SCT) can induce hematologic responses, organ responses and lead to improvement in survival in selected patients with AL (immunoglobulin light chain) amyloidosis.[1] The depth of hematologic response, in particular the achievement of complete hematologic response (CR), has been shown to be predictive of clinical response, quality of life and improvement in survival.[1-3] The median survival of patients achieving hematologic CR after HDM/SCT in a landmark analysis of patients alive at one year following treatment exceeds ten years compared to 50 months for those not achieving a hematologic CR.[4]

The proteasome inhibitor bortezomib has been approved for treatment of myeloma. Recent studies demonstrate high response rates when bortezomib is used in combination with oral melphalan and prednisone.[5] While the mechanism of action is still not completely understood, *in vitro*, bortezomib senstitizes myeloma cells to DNA-damaging agents such as melphalan, and overcomes chemoresistance.[6] It also acts upon the bone marrow microenvironment, inhibiting nuclear factor-κB activation in bone marrow stromal cells. This leads to a reduction in interleukin-6 production and enhanced apoptosis of myeloma cells.[7] Recently, bortezomib has been incorporated into HDM conditioning for SCT in myeloma.[8] Pre-clinical and phase I/II data have suggested that the optimal timing of administration of a single dose of bortezomib is 24 h after melphalan.[6,9]

Because hematologic CR is a critical determinant of treatment outcome following HDM/SCT, we hypothesized that the addition of bortezomib to HDM/SCT could increase hematologic CR rates in patients with AL amyloidosis. This hypothesis led us to conduct a prospective feasibility pilot study of bortezomib-HDM/SCT for the treatment of AL amyloidosis (*ClinicalTrials.gov: NCT00790647*). The objective of this