

TO THE EDITOR:

Expression of activated B-cell gene signature is predictive of the outcome of follicular lymphoma

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Gene expression profile (GEP)-defined cell of origin-based classification enables the molecularly and clinically distinct disease subcategorization in diffuse large B-cell lymphoma (DLBCL), that is, prognostically favorable germinal center B-cell (GCB) and unfavorable activated B-cell (ABC) types.¹ GCB-DLBCL frequently harbors *BCL2* gene rearrangement and mutations of epigenetics-related genes such as *CREBBP*, *EZH2*, and *KMT2D*, whereas ABC-DLBCL is characterized by the constitutive activation of B-cell receptor and NF- κ B signaling pathways by various gene abnormalities such as mutations of *CD79A/B*, *MYD88*, and *CARD11* and the loss of *TNFAIP3*.^{2,3}

Despite the overall improvement in prognosis with the advent of immunochemotherapeutic approaches over the last 2 decades,^{4,5} follicular lymphoma (FL) still remains incurable, and patients with early progression of this disease have a poor prognosis.⁶ The origin of FL has generally been considered as GCB, and the pattern of molecular abnormalities of FL tumors frequently overlaps with that of GCB-DLBCL.⁷ However, recent epigenetic analysis and enhancer profiling revealed the existence of 2 molecularly defined subtypes of FL tumors, namely, germinal center centrocyte-like and in vitro-activated plasmablast-like,⁸ but the clinical significance of these classifications remains unknown. Thus, we investigated the association between prognosis and the GEP of FLs using publicly available GEP data sets, and we developed a new molecular signature-based prognostically relevant classification system for FL.

First, we constructed new lists of GCB and ABC signature genes since hierarchical clustering with the previously established gene sets for GCB and ABC failed to classify FL because of its heterogeneous expression patterns (supplemental Figure 1). We used GSE15271 and E-MEXP-2360 data sets from nontumoral human lymphocytes to build gene sets GCB-sig (100) (top 100 highly expressed genes in centrocytes and centroblasts) and ABC-sig (100) (top 100 highly expressed genes in activated B cells) and GCB-sig ($q < 0.001$) and ABC-sig ($q < 0.001$), which consisted of a wider range of GCB-sig and ABC-sig genes (supplemental Methods; supplemental Figure 2). To examine the expression pattern of GCB-sig genes, we performed unbiased hierarchical clustering of GCB-sig (100) genes in the exploratory data (GSE93261) and identified a distinct subtype that showed uniformly high GCB-sig gene expression (supplemental Figure 3). Given that another cluster represents the heterogeneous expression pattern on the heatmap (supplemental Figure 3), we classified all tumors by quartiles of mean z-score of GCB-sig (100) genes and observed a uniform pattern of GCB-sig gene expression (Figure 1A). Moreover, the tumors in the highest quartile (Q1) significantly expressed lower ABC-sig (100) genes than the lowest quartile (Q4) ($P = 3.96 \times 10^{-5}$; Student *t* test) (Figure 1B). Because the GCB-sig gene sets used in the above analyses might contain redundant genes, we aimed to develop a generally applicable and robust classifying tool for FL. We built new gene sets that consisted of a wide range of GCB-sig and ABC-sig genes and selected the 33 important classifying genes (26 GCB-sig and 7 ABC-sig genes) from GCB-sig ($q < 0.001$) genes and ABC-sig ($q < 0.001$) genes. By comparing Q1 and Q4 using the Boruta method, we were able to construct the 33-gene expression-based score system, and we defined tumors with under the cutoff value of 0 as GCB-like FLs and those over the cutoff value of 0 as ABC-like FLs (supplemental Methods). In the exploratory data, the tumors with a lower score showed a distinct GCB-like pattern (Figure 1C). In validation data 1 (GSE62246) (supplemental Figure 4;

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Requests for data sharing may be submitted to Junya Kuroda (junkuro@koto.kpu-m.ac.jp).

The full-text version of this article contains a data supplement.

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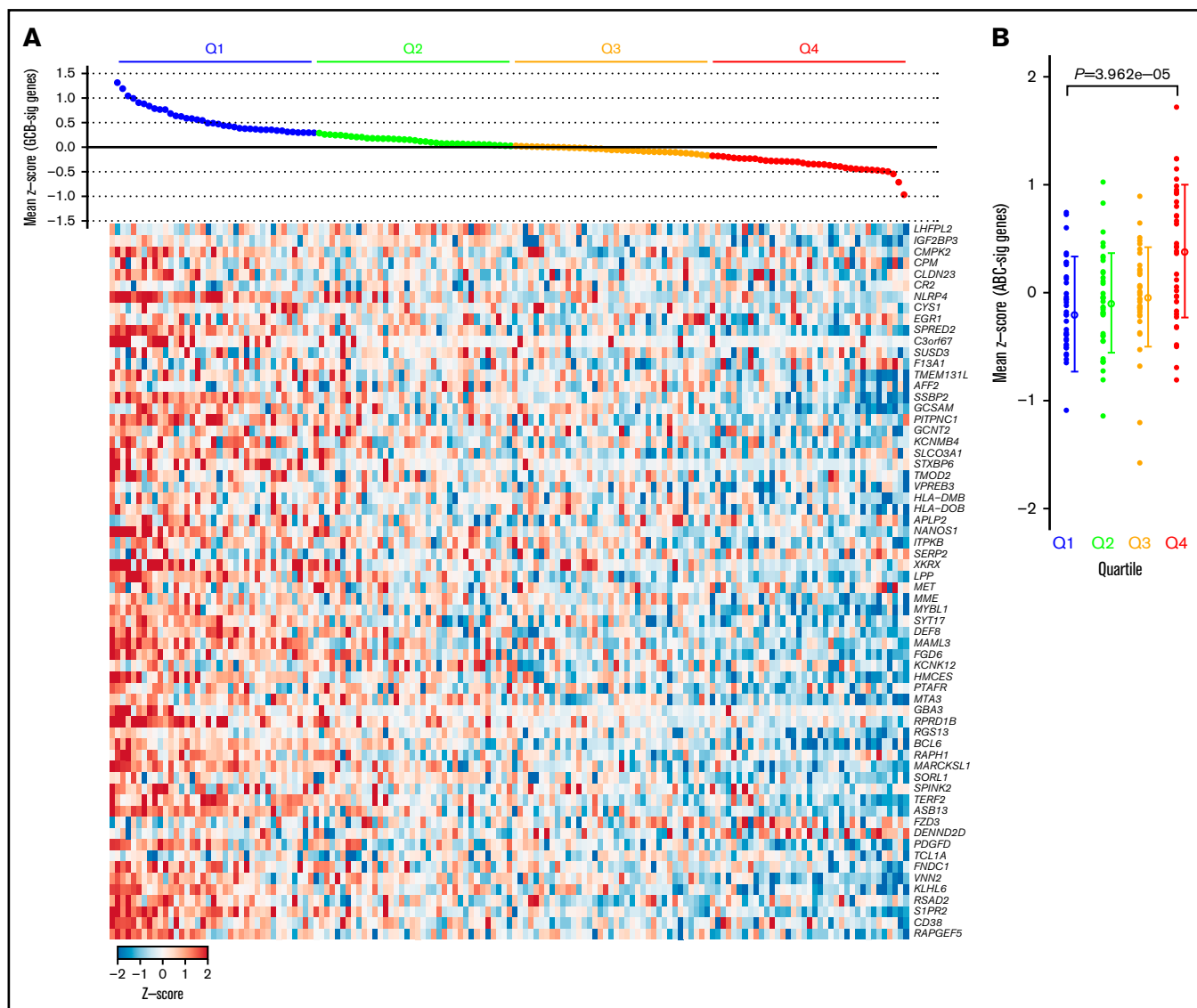


Figure 1. Expression analyses of GCB-sig and ABC-sig genes in FLs. (A) Heatmap of GCB-sig gene expression in the exploratory data (GSE93261). All tumors are sorted by mean z-scores of GCB-sig (100) genes and classified by quartile of mean z-scores of GCB-sig (100) genes. (B) Comparisons of mean z-score of ABC-sig (100) genes among quartiles of mean z-scores of GCB-sig (100) genes. Open circles and error bars indicate means and standard deviations, respectively. (C) The center heatmap represents all tumors in the exploratory data (GSE93261) sorted by the predictor scores. The left heatmap represents normal activated B cells (actB), centrocytes (CC), and centroblasts (CB). Right panel: scores of each classifying gene; top panel: scores of each tumor; bottom panel: quartile classification according to panel A by color. Blue, first quartile (Q1); green, Q2; orange, Q3; red, Q4.

supplemental Table 1), we evaluated the concordance between our score-based classification and the previously represented epigenetics-based subtypes⁸ and found that our classification system had good predictive value, with area under the curve of 0.889 below the cutoff value of 0.04. This 33-gene expression-based score could classify GCB-like and ABC-like tumors (supplemental Figures 5 and 6). GCB-like FLs frequently harbored *EZH2* and *MEF2B* mutations in validation data 2 (GSE66166), whereas ABC-like FLs frequently lacked t(14;18) in validation set 4 (GSE16131) and harbored loss of 6q13 in validation set 5 (GSE53820 and E-TABM-930) (supplemental Figures 5-7). In addition, gene set enrichment analysis comparing ABC-like and GCB-like FLs in the exploratory data showed that GCB-like FLs highly

expressed Blimp-1-repressed genes, and ABC-like FLs highly expressed IRF4-activated genes associated with ABC-DLBCL (supplemental Figure 8; supplemental Table 2).^{9,10}

The univariable and multivariable analyses for failure-free survival (FFS) and overall survival (OS) revealed that ABC-like FLs were associated with worse prognoses than were GCB-like FLs (Figure 2; supplemental Table 3). Moreover, integration of the expression-based classification and Follicular Lymphoma International Prognostic Index (FLIPI) category revealed patients with remarkably poor outcome (supplemental Figure 9). Although the number of patients without t(14;18) was higher in ABC-like FLs, the absence of t(14;18) was

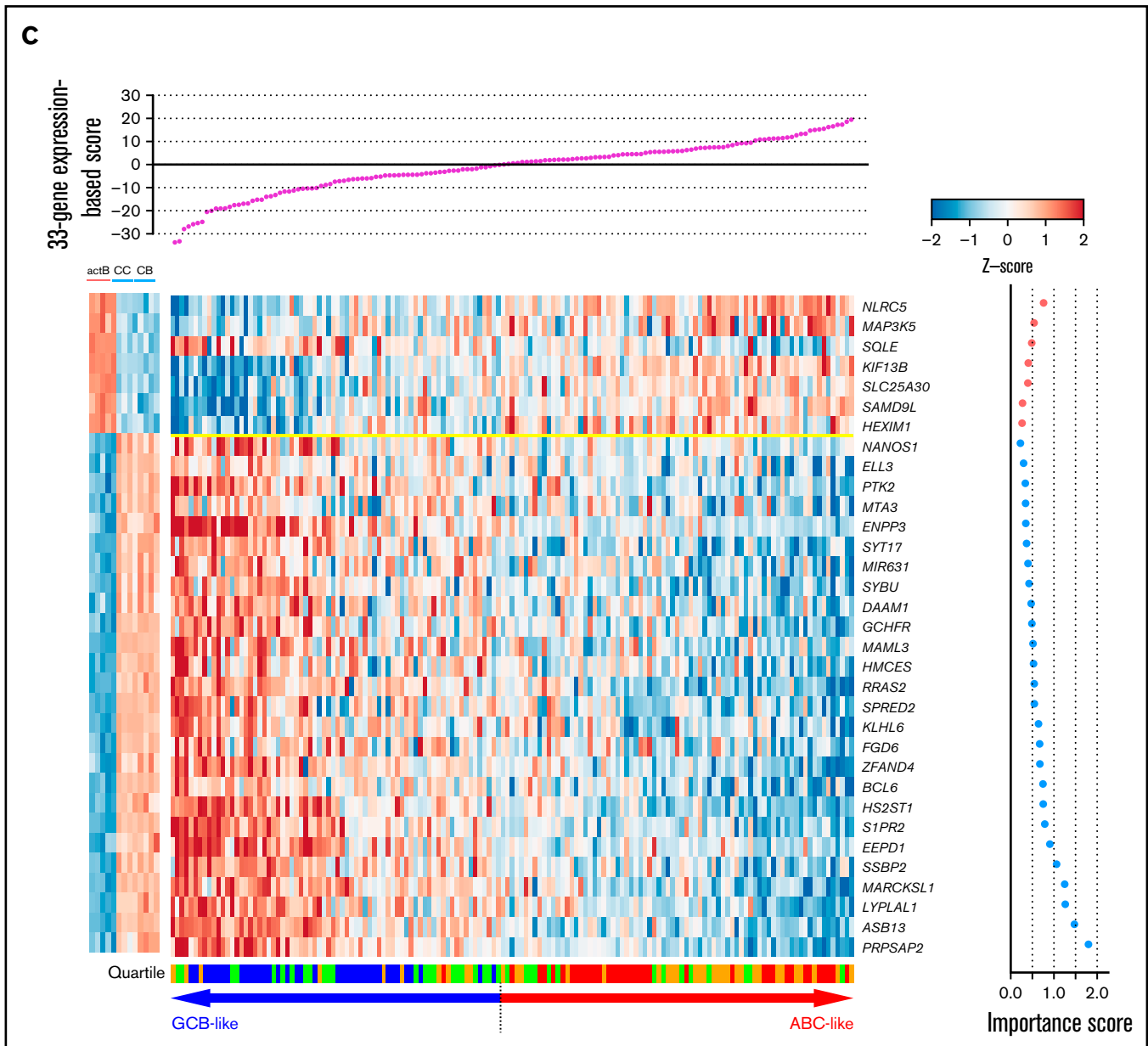


Figure 1. (continued)

not associated with OS in validation set 4 (GSE16131) (log-rank test $P = .25$). Despite utility as a prognostic factor, this novel classification was not predictive for transformation of FL in validation set 5 (GSE53820) (supplemental Figure 7).

The Lymphoma Study Association group recently developed a 23-gene expression-based predictor model for progression-free survival in patients with FL who have a high tumor burden¹¹; however, those 23 genes are functionally heterogeneous. Several studies have suggested a close association between therapeutic sensitivities and cell of origin–defined subtypes in DLBCL.^{12,13} By contrast, there have been no or few if any clinical trials for FL in which therapeutic efficacy was assessed by GEP of tumor cells. In this study, we developed the novel 33-gene expression-based score for FL on the basis

of the gene expression signature of nontumoral B lymphocytes, and we found associations between ABC-like FLs and poor OS and FFS across different data sets. Thus, the current 33-gene expression-based model is expected to help exploit the rational selection of targeted therapy for FL.

Although it is widely recognized that FL tumors share relatively uniform molecular features and develop from GCB, previous analysis proposed the presence of a distinct FL subtype with a molecular feature resembling plasmablasts.⁸ Our results also suggest that a portion of FLs is biologically different from typical FLs that originate from the germinal center, which have close molecular similarity to DLBCL. Like EZB-DLBCL (with *EZH2* mutations and *BCL2* translocations, most of which consist of GCB-DLBCL), GCB-like FLs

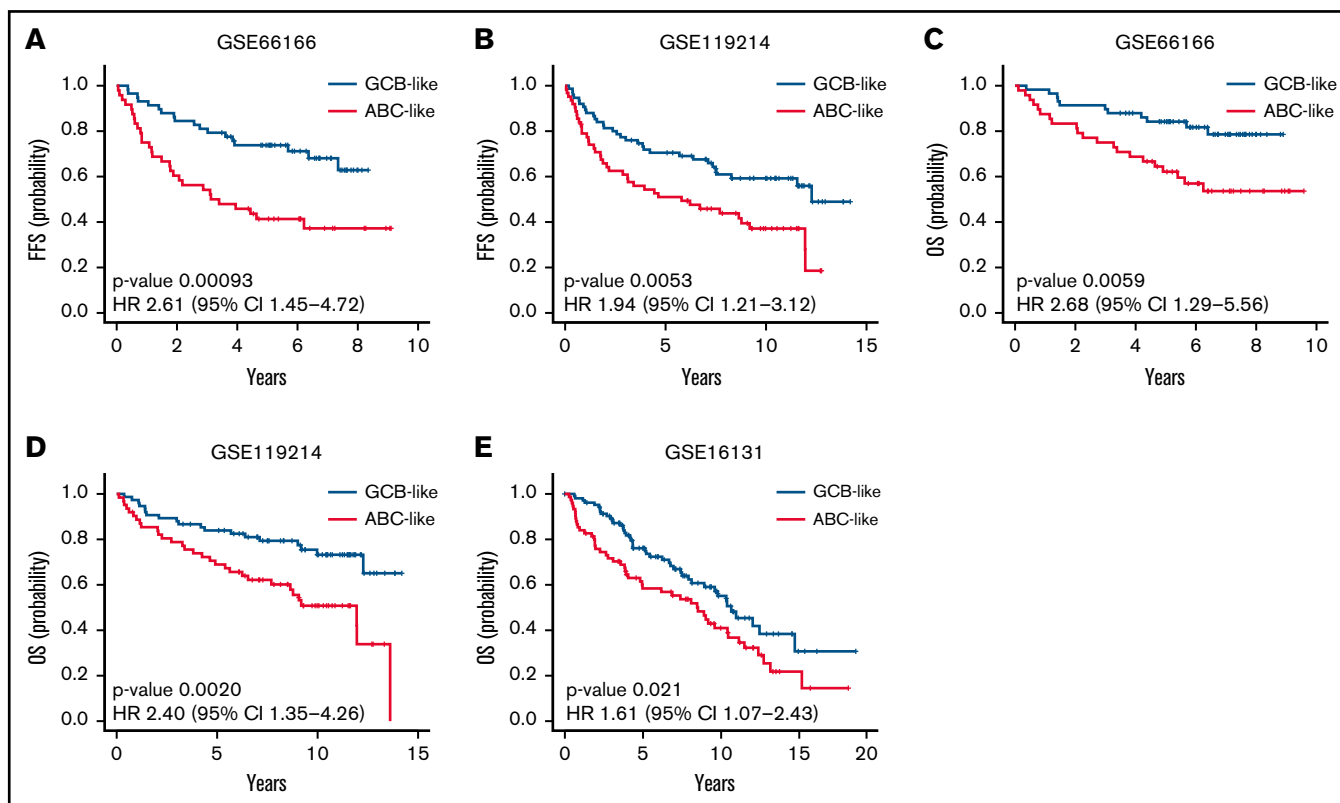


Figure 2. Comparison of failure-free survival (FFS) and OS for the newly developed 33-gene expression-based scores in the validation data sets. FFS of GSE66166 (A) and GSE119214 (B). OS of GSE66166 (C), GSE119214 (D), and GSE16131 (E), comparing GCB-like (blue) and ABC-like (red) FLs. CI, confidence interval; HR, hazard ratio.

frequently harbored *EZH2* and *MEF2B* mutations and highly expressed IRF4-repressed genes. Conversely, ABC-like FLs frequently lacked *BCL2* translocations and highly expressed IRF4-upregulated genes (supplemental Figure 10).¹⁴

This study has several limitations. First, information about treatment regimens was not available in some of the data sets used in this study. Presumably, some samples are from the prirituximab era. Validating the prognostic value of the current model in the cohort treated with the latest standard immunochemotherapeutic regimens, such as those that include bendamustine, lenalidomide, or obinutuzumab, is warranted. Second, the proportions of GCB-like and ABC-like FLs are different from those of previously described subtypes, namely centrocyte-like type and in vitro-activated plasmablast-like type.⁸ Integrated analysis of GEP and epigenetic profiling with a large sample size would further verify the accuracy of the current classification system. Third, the data used in this study were not uniform in terms of the microarray chips and the methods used to purify tumor cells and preserve samples. Moreover, the microarray approach is not easily applicable in daily clinical practice. To use our classification system in daily practice, a standardized alternative method to our novel 33-gene expression-based score needs to be developed.

In conclusion, we developed a novel classification system of 33-gene expression for FL and demonstrated poor prognosis for ABC-like FL. Further research to identify promising therapeutic molecular targets for ABC-like FL is required.

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Contribution: T.T. designed the research, analyzed the data, and drafted the manuscript; Y.T. and M.N. analyzed the data; and K.T. and J.K. designed and supervised the research.

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