

Editorial

Cell-of-Origin in Diffuse Large B-Cell Lymphoma: Are the Assays Relevant for Clinical Use?

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma worldwide and has highly heterogeneous clinical outcomes with conventional chemoimmunotherapy. Although the course of the disease seems to be aggressive, more than half of patients can be cured by rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP)/R-CHOP-like regimens. These standard regimens induce patients into complete remission, 5-year progression-free survival (PFS) and 5-year overall survival (OS) of 75%, 54% and 58%, respectively.¹ The international prognostic index (IPI) series including IPI, revised IPI (R-IPI) and enhanced IPI (NCCN-IPI) are simple clinical tools used to predict patients with DLBCL outcomes. However, these prognostic indices are only moderately correlated with the OS for Asian patients treated with standard chemoimmunotherapy.² Based on gene expression profile (GEP) analysis, the molecular classification of DLBCL discriminating germinal center B-cell like (GCB) from activated B-cell like (ABC) remains a cornerstone for DLBCL biology and is proven to be correlated with clinical outcomes in patients treated with chemoimmunotherapy.

Molecular diffuse large-B cell lymphoma subtypes, prognostic implications and treatment decisions

The WHO classification of lymphomas has continuously changes with the new knowledge of the pathophysiology, phenotypical and/or genetic characteristics of lymphoma cells and cell-of-origin (COO). The concept of COO aims to assign every lymphoid neoplasm to its possibly closest normal counterpart, but not the case for DLBCL which was highly heterogeneous to sufficiently

characterize. The gold standard method for COO is GEP analysis of RNA from fresh frozen tissue (FFT) using microarray technology. In contrast to formalin-fixed paraffin-embedded tissue (FFPET), GEP analysis is an expensive, time-consuming procedures and not easy to establish nationwide in Thailand. Seventeen years ago, a transcriptomic analysis of FFT of DLBCL was reported based on an analysis of more than 17,000 probes covering genes involved in germinal center biology, cancer biology, lymphoma and normal lymphocyte biology.³ As a result, there are 2 subtypes of DLBCL-GCB and ABC using GEP analysis. Importantly, the GCB/ABC signature was highly correlated with the OS of patients treated with CHOP regimen, while the ABC subtype was associated with the worse outcome. Later, this early work was further refined with a large cohorts using GEP based on 100 genes and led to the distinction of the following 3 groups: GCB, ABC and unclassifiable DLBCL.⁴ Importantly, this study confirmed the differences in the oncogenetic mechanisms between ABC and GCB DLBCL, and the strong prognostic value of this classifier.⁴ Either in frontline therapy and in relapsed DLBCL, recent studies have confirmed that the worse prognosis associated with the ABC subtype was maintained in the rituximab era.⁵⁻⁷ In first-line therapy, young patients with non-GCB DLBCL receiving intensive chemoimmunotherapy have better survival as compared with patients treated with conventional R-CHOP.⁷ Moreover, a predictive value of the COO classification potentially altered therapeutic choice in relapsed DLBCL patients because GCB DLBCL was associated with a better survival rate with R-DHAP (rituximab, cisplatin and cytarabine) than R-ICE (rituximab, carboplatin, ifosfamide and etoposide).⁶

The predictive and prognostic potential of DLBCL classification urged the development of surrogate markers to easily determine the COO in clinical practice. Hans and colleagues performed the first study to translate the GEP signature of DLBCL for routine purposes based on immunohistochemistry (IHC) on FFPE. Hans algorithm, one of the most popular IHC-based determination of COO, was used to describe GCB DLBCL and ABC DLBCL as CD10-positive or BCL6-positive and MUM1-negative and CD10-negative and MUM1-positive, respectively.⁸ The concordance between the IHC-based assay and GEP analysis in the validation cohort was 79%.⁸ In order to improve the Hans algorithm, Choi and colleagues (Choi algorithm), Meyer and colleagues (Tally algorithm), and Visco and colleagues (Visco algorithm), proposed different combinations of GCB markers (BCL6, CD10, GCET1 and LMO2) and ABC markers (MUM1/IRF4 and FoxP1) to discriminate between DLBCL molecular subtypes (Figure 1).⁸⁻¹¹ The reported concordance between IHC-based assay and GEP analysis in the validation cohort according to Choi, Tally and Visco algorithm was 88%, 93% and 93%, respectively.⁹⁻¹¹ However, there were discrepancies in the clinical outcomes predicted by these different classifiers and all of them were not good enough to classify DLBCL compared with the gold standard GEP classifier in further studies (Table 1).¹²⁻¹⁴ One explanation for these disappointing results may be

the poor reproducibility of the quantitative assessment of the immunostaining.¹⁵ In addition, these algorithms can only recognize 2 categories of lymphoma and fail to identify the unclassifiable cases based on GEP analysis.

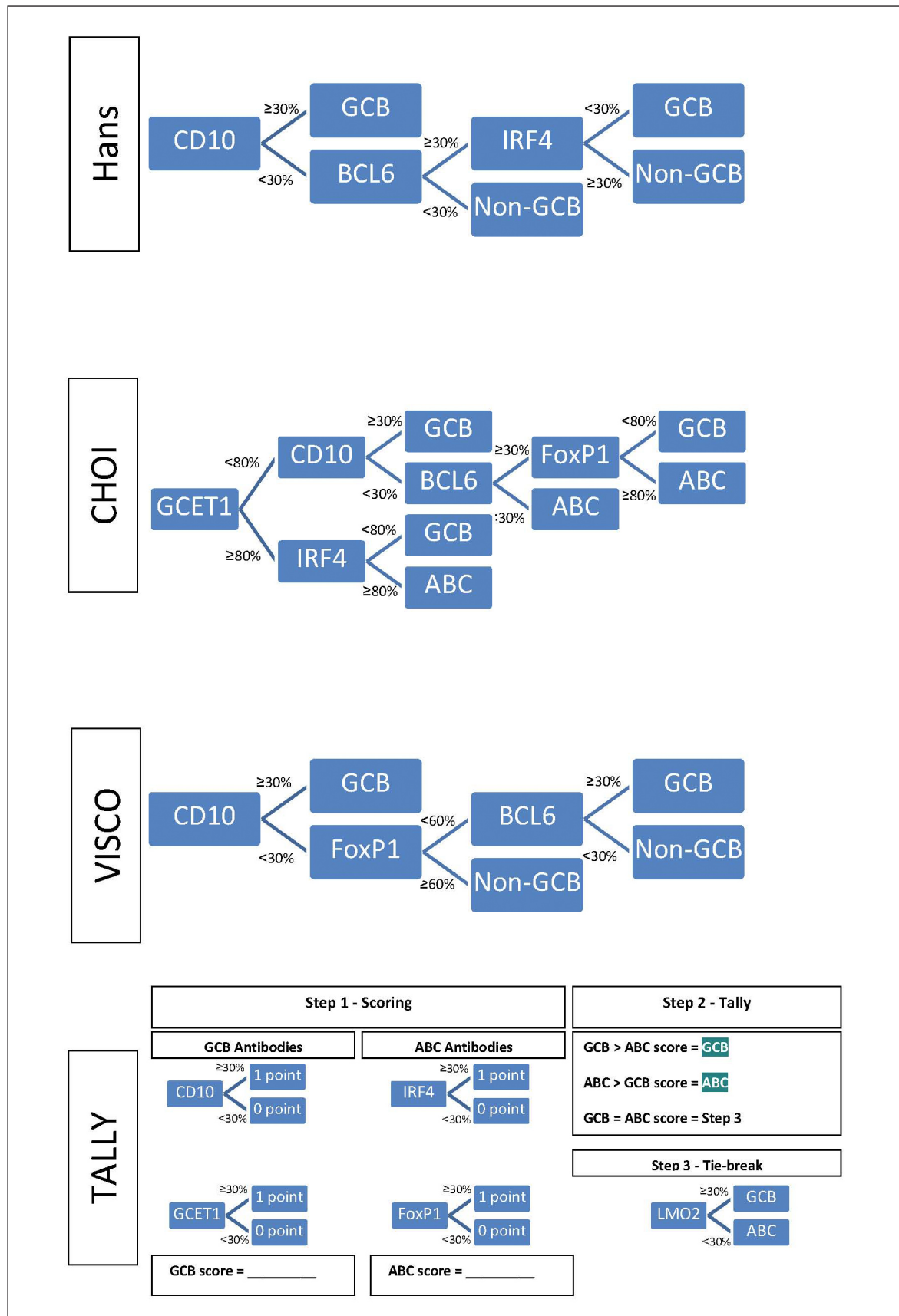
In disagreement with previous studies, Noiwanakul and colleagues have conducted a retrospective study to evaluate the prognostic implication of Hans algorithm and demonstrated that the non-GCB subgroup was independently associated with significantly shorter OS and PFS in Thai patients with DLBCL receiving R-CHOP regimen as compared with GCB subgroup.^{15,16} However, Hans algorithm is currently being used to provide the patient selection for a phase III, randomized, controlled trial assessing the efficacy of adding ibrutinib to R-CHOP in non-GCB DLBCL (ClinicalTrials.gov Identifier NCT01855750).

Conclusion

In spite of the comprehensive studies defining the assays and the wide availability of IHC, the accumulated evidence indicated that IHC-based methods are not ready to guide clinical decision at this time. The antibodies, laboratory techniques and agreement on scoring of IHC-based assay needed to be standardized to improve its reproducibility and accuracy for clinical management.

Table 1 Various immunohistochemistry-based algorithms which were trained against gene expression profiling are shown.

Algorithm	Accuracy (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Hans	65-86	86-90	52-82	55-82	83-90
Choi	59-88	84-89	40-100	52-88	77-90
Visco	93	92	93	92	93
Tally	65-93	80-90	54-86	55-87	79-99



CD10, cluster of differentiation 10; BCL6, B-cell lymphoma 6; GCB, germinal center B-cell like; IRF4, interferon regulatory factor 4; GCET1, germinal center B-cell expressed transcript-1; ABC, activated B-cell like; FoxP1, forkhead box P1; LMO2, LIM domain only 2; DLBCL, diffuse large B-cell lymphoma

Figure 1 The algorithms are used to describe GCB DLBCL and non-GCB DLBCL

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