Integration of COO into the clinical CNS International Prognostic Index could improve CNS relapse prediction in DLBCL

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CNS relapse in DLBCL in the GOYA study

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1. High CNS-IPI score and ABC/unclassified COO subtypes were independent risk factors for CNS relapse in DLBCL.
2. Combining CNS-IPI score and COO improved identification of DLBCL patients with different CNS relapse risks.

Abstract

Central nervous system (CNS) relapse carries a poor prognosis in diffuse large B-cell lymphoma (DLBCL). Integrating biomarkers into the CNS International Prognostic Index (CNS-IPI) risk model may improve identification of patients at high risk of developing secondary CNS disease. CNS relapse was analyzed in 1,418 DLBCL patients treated with obinutuzumab or rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy in the phase III GOYA study (NCT01287741). Cell-of-origin (COO) was assessed using gene expression profiling. BCL2 and MYC protein expression were analyzed by immunohistochemistry. The impact of CNS-IPI, COO, and BCL2/MYC dual-expression status on CNS relapse was assessed using a multivariate Cox regression model (data available in n = 1,418, n = 933, and n = 688, respectively). High CNS-IPI score (hazard ratio [HR], 4.0; 95% confidence interval [CI], 1.3–12.3; *P* = .02) and activated B-cell–like (ABC) (HR, 5.2; 95% CI, 2.1–12.9; *P* = .0004) or unclassified COO subtypes (HR, 4.2; 95% CI, 1.5–11.7; *P* = .006) were independently associated with CNS relapse. BCL2/MYC dual-expression status did not impact CNS relapse risk. Three risk subgroups were identified according to the presence of high CNS-IPI score and/or ABC/unclassified COO (CNS-IPI-C model): low risk (no risk factors, n = 450 [48.2%]); intermediate risk (one factor, n = 408 [43.7%]); and high risk (both factors, n = 75 [8.0%]). Two-year CNS relapse rates were 0.5%, 4.4%, and 15.2% in respective risk subgroups. Combining high CNS-IPI and ABC/unclassified COO improved CNS relapse prediction and identified a patient subgroup at high risk of developing CNS relapse.

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Introduction

Central nervous system (CNS) relapse is a rare, usually fatal, event in diffuse large B-cell lymphoma (DLBCL); median overall survival (OS) after its occurrence is 3.5 to 7 months.[1,2] Addition of rituximab (R) to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves outcomes in DLBCL patients;[3,4] however, its impact on the incidence of secondary CNS disease remains unclear, with some studies demonstrating reduced CNS relapse risk in DLBCL patients treated with R-CHOP vs CHOP[5,6] and others showing similar CNS relapse rates.[7] Reliable identification of patients at higher risk of developing secondary CNS disease is needed. Several clinical prognostic models have been proposed.[1,2,8] The CNS International Prognostic Index (CNS-IPI) model is the most recently developed,[1] and was built using a large dataset of patients with aggressive B-cell lymphomas (80% DLBCL), who were enrolled in studies from the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) and MabThera International Trial (MInT), and was successfully validated in population-based DLBCL cohorts.[9,10] The model includes the IPI risk factors plus involvement of the kidneys and/or adrenal glands. Implementation of biomarkers into the CNS-IPI model may improve identification of patients with high risk of CNS relapse.[9]

DLBCL represents a biologically heterogeneous disease with germinal center B-cell–like (GCB) and activated B-cell–like (ABC) subtypes, each arising from different non-malignant lymphoid counterparts.[11] DLBCL cell-of-origin (COO) subtypes harbor specific genetic abnormalities;[12-14] for example, GCB DLBCL is characterized by frequent translocations of the *BCL2* gene and loss of *PTEN*, while ABC DLBCL is characterized by biallelic loss of the *CDKN2A* gene, which encodes proteins implicated in regulation of the cell cycle (p16INK4A) and p53 (ARF), and chronically

active B-cell receptor and NFκB signaling.[12,15-18] The impact of COO subtype on prognosis has been confirmed in several studies, with the ABC subtype predicting worse outcomes.[19,20] ABC DLBCL was also shown to be the most common COO subtype in primary CNS lymphomas.[21] Data are limited on the association of COO subtype with the risk of secondary CNS disease in DLBCL, with only one retrospective study published to date. Savage and colleagues showed that ABC (or non-GCB) DLBCL is associated with higher CNS relapse risk.[9] In a multivariate analysis including COO subtype, dual-expression status of BCL2 and MYC proteins, and CNS-IPI, only high CNS-IPI score and BCL2/MYC dual-expression were significantly associated with CNS relapse risk.[9]

GOYA is a multicenter, randomized, phase III trial (NCT01287741) investigating the efficacy and safety of obinutuzumab (G) or R plus CHOP in patients with previously untreated DLBCL. After a median observation time of 29.0 months, there were no significant differences between G-CHOP and R-CHOP for progression-free survival (PFS) and OS;[22] 3-year investigator-assessed PFS rates were 70% and 67%, respectively. Patients with GCB DLBCL demonstrated better outcomes than those with ABC or unclassified DLBCL, with 3-year PFS rates of 75%, 59%, and 63%, respectively. Using data from GOYA, we aimed to evaluate the impact of distinct COO subtypes and dual-expression of BCL2 and MYC proteins on CNS relapse risk.

Methods

Patients, treatment, and clinical assessments

The GOYA study design is described in full elsewhere.[22] Patients had previously untreated, histologically documented, CD20-positive DLBCL and an IPI score of ≥2, an IPI score of 1 (if age ≤60 years, with or without bulky disease), or an IPI score of

0 (with bulky disease [one lesion ≥7.5 cm]). Patients with CNS involvement at diagnosis were excluded.

Patients received eight 21-day cycles of G or R plus six to eight cycles of CHOP chemotherapy. CNS prophylaxis with intrathecal chemotherapy was recommended to be given according to institutional practice. No systemic CNS-directed prophylaxis was administered.

Staging investigations included computed tomography (CT) scan and bone marrow biopsy. Baseline lumbar puncture was recommended in patients with high-risk disease or with one or more of the following sites of involvement: paranasal sinuses, testicular, parameningeal, periorbital, paravertebral, or bone marrow. CNS relapse was diagnosed according to institutional practice via imaging (magnetic resonance imaging or CT scan), and/or presence of malignant cells in cerebrospinal fluid or affected tissue. The protocol was approved by the ethics committees of participating centers. All patients provided written informed consent.

COO, immunohistochemical (IHC), and fluorescence in-situ hybridization (FISH) analyses

COO classification was performed by a central laboratory based on gene-expression profiling using the NanoString Lymphoma Subtyping Research-Use-Only assay (NanoString Technologies, Seattle, WA). IHC analysis using BCL2 (clone 124) and MYC (clone Y69) assays (Ventana Medical Systems, Tucson, AZ) was conducted on slides cut from diagnostic formalin-fixed, paraffin-embedded (FFPE) blocks. Cut slide stability was not considered for selection of tissue sections for analysis. BCL2 protein expression was assessed according to the percentage of tumor cells with BCL2 expression and staining intensity; positivity was defined as moderate or strong

staining in ≥50% of tumor cells. MYC positivity was defined as expression in ≥40% of tumor cells. IHC analyses were conducted in a central laboratory (Hematogenix, Chicago, IL). FISH was performed in a central laboratory (HistoGeneX, Antwerp, Belgium) on the diagnostic FFPE tissue sections using Vysis LSI dual-color break-apart probes for *BCL2* and *MYC* rearrangement detection, as previously described.[23]

Targeted next-generation sequencing (NGS)

Genomic DNA was extracted from diagnostic FFPE tissue sections containing ≥20% tumor cells. Samples were submitted to a central laboratory (Foundation Medicine, Cambridge, MA) for NGS-based genomic profiling. Adaptor-ligated DNA underwent hybrid capture for all coding exons of 465 cancer-related genes (FoundationOne Heme platform). Captured libraries were sequenced to a median exon coverage depth of >500× (DNA) using Illumina sequencing, and resultant sequences were analyzed for base substitutions, small insertions and deletions (indels), copy number alterations (focal amplifications and homozygous deletions), and gene fusions/rearrangements, as previously described.[24] Frequent germline variants from the 1000 Genomes Project (dbSNP142) were removed. To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥ 5% mutant allele frequency (MAF), indels with a ≥ 10% MAF with ≥ 99% accuracy, and fusions occurring within baited introns/exons with > 99% sensitivity.[24] Known confirmed somatic alterations deposited in the Catalogue Of Somatic Mutations In Cancer (COSMIC v62) are called at allele frequencies ≥ 1%.[25] NGS-based genomic profiling was performed in a subset of patients (617 of 1,418) who provided an optional

written informed consent; data that passed the quality check criteria were evaluable in 499 of 617 patients.

Statistical analysis

The event-specific, cumulative incidence of CNS relapse and time to CNS relapse were estimated with Kaplan-Meier statistics. The impact of variables of interest (CNS-IPI, COO, BCL2/MYC dual-expression status, *CDKN2A* alteration, and the GOYA study randomization stratification factors—number of planned chemotherapy cycles, geographical region) on CNS relapse was assessed using univariate and multivariate Cox regression models. In these models, the endpoint of interest was time to CNS relapse, defined through the manual review of patients with disease progression or a death event at the time of the primary analysis cut-off (29 April 2016). The significance level, used consistently, was 5%; all tests are two-sided. No multiplicity adjustment was performed in order to avoid loss of power due to the low number of events, which is a structural limitation of such rare phenomena. The R statistical software package version 3.4.0,[26] together with RStudio version 1.0.153,[27] was used for all analyses.

Results

Overall, 1,418 DLBCL patients, randomized and treated with G-CHOP (n = 706) or R-CHOP (n = 712) in GOYA, were analyzed for CNS relapse occurrence. Baseline characteristics are shown in Table 1. According to CNS-IPI score, 279 (19.7%) patients were categorized as being at low risk (0 to 1), 894 (63.0%) at intermediate risk (2 to 3), and 245 (17.3%) at high risk (4 to 6) of developing CNS relapse. COO was available for 933 patients, of whom 540 (57.9%), 243 (26.0%), and 150 (16.1%)

were classified as GCB, ABC, and unclassified DLBCL, respectively. Both COO and BCL2/MYC protein expression were available in 688 patients; 295 (42.9%) were BCL2/MYC dual-expressers. More patients with ABC DLBCL were BCL2/MYC dual-expressers compared with GCB or unclassified (136 [70.5%] vs 117 [30.7%] vs 42 [36.8%], respectively; Table 2).

Incidence and outcome of CNS relapse

After a median observation of 29.0 months (interquartile range, 24.5–37.4), 38 (2.7%) of the 1,418 patients developed CNS relapse (17 patients treated with chemotherapy only, 6 with chemotherapy and radiotherapy, 4 with radiotherapy only, 6 received no treatment; data not available in 5 patients); 37 of these had either radiological signs of CNS relapse and/or infiltrated CSF. In one patient, CNS relapse (intraocular) was diagnosed via cytological evaluation of corpus vitreum. Most CNS relapses were localized in the brain parenchyma (parenchymal only, n = 27 [71.1%]; leptomeningeal only, n = 6 [15.8%]; parenchymal and leptomeningeal, n = 3 [7.9%]; intraocular, n = 1 [2.6%], and data not available, n = 1 [2.6%]). Median time to CNS relapse was 8.5 months (range, 0.9–43.5). The majority (34 [89.5%]) of CNS relapses occurred within 2 years of randomization. The 2-year CNS relapse rate for the whole cohort was 2.8%. Twenty-four (63%) of 38 patients with CNS relapse were dead at the time of the analysis; median survival after CNS relapse was 5.9 months. According to CNS-IPI, 10.5% of patients with CNS relapse were categorized as low-risk, 42.1% as intermediate-risk, and 47.4% as high-risk. Two-year CNS relapse rates were 0.8% (95% CI, 0.0–1.9), 1.9% (95% CI, 0.9–2.9), and 8.9% (95% CI, 4.7–12.9) for the low-, intermediate-, and high-risk CNS-IPI subgroups, respectively (Figure 1A).

Treatment arm and prophylaxis with intrathecal chemotherapy and CNS relapse risk

The number of CNS relapses was similar in the G-CHOP and R-CHOP arms (20 vs 18, respectively), with no impact of treatment arm on the incidence of CNS relapse (hazard ratio [HR], 1.13; 95% CI, 0.60–2.15; *P* = .70). Overall, 140 (9.9%) of 1,418 patients received intrathecal methotrexate or cytarabine or a combination of both as CNS relapse prophylaxis. Within the low-, intermediate-, and high-risk CNS-IPI groups, 16 (5.7%) of 279, 94 (10.5%) of 894, and 30 (12.2%) of 245 patients received intrathecal CNS relapse prophylaxis, respectively (Supplemental Table S1). Two-year CNS relapse rates were not different between patients who did or did not receive CNS relapse prophylaxis (2.8% vs 2.6%). Similarly, the number of CNS relapses was not different in patients treated with or without prophylaxis in any of the CNS-IPI categories (0.0% vs 0.9%, 1.3% vs 2.0%, and 8.5% vs 9.0% for the low-, intermediate-, and high-risk CNS-IPI subgroups, respectively; Supplemental Table S1).

COO and BCL2/MYC dual-expression status and CNS relapse risk

In patients with COO available (n = 933, 30 CNS-relapse events; Supplemental Table S2), 2-year CNS relapse rates were 1.4% (95% CI, 0.0–3.2), 2.2% (95% CI, 0.9–3.5), and 9.6% (95% CI, 4.5–14.5) for the low-, intermediate-, and high-risk CNS-IPI subgroups, respectively (Figure 1B). On univariate analysis, patients with ABC and unclassified DLBCL had significantly higher CNS relapse risk than those with GCB DLBCL (HR, 5.2; 95% CI, 2.1–12.7; *P* = .0003; and HR, 4.2; 95% CI, 1.5–11.7; *P* = .005; respectively). Two-year CNS relapse rates were 6.9%, 4.8%, and 1.3% for patients with ABC, unclassified, and GCB DLBCL, respectively. There was

no significant association between BCL2/MYC dual-expression and the risk of CNS relapse on univariate analysis (HR, 1.5; 95% CI, 0.7–3.5, *P* = .3196; 2-year CNS relapse rate: dual-expressers 4.0% vs non-dual-expressers 2.2%; n = 688). In a multivariate analysis on the COO-available population (n = 933), ABC (HR, 5.2; 95% CI, 2.1–12.9; *P* = .0004) and unclassified COO subtype (HR, 4.2; 95% CI, 1.5–11.7; *P* = .006), and high CNS-IPI (HR, 4.0; 95% CI, 1.3–12.3; *P* = .02) were associated with greater CNS relapse risk (Table 3). In a multivariate analysis on the population with COO and BCL2/MYC dual-expression status available (n = 688, 22 CNS-relapse events; Supplemental table S2), there was no impact of BCL2/MYC dual-expression (HR, 0.8; 95% CI, 0.3–2.1; *P* = .69) on CNS relapse risk, while ABC and unclassified COO subtype remained significantly associated with higher CNS relapse risk (Table 4). In this population, high CNS-IPI score was not significantly associated with CNS relapse risk, although a trend for greater risk was observed (HR, 2.8; 95% CI, 0.8–9.4; *P* = .10).

Overall, 560 (39.5%) of 1,418 patients had FISH results available. Twenty patients (3.6%) harbored both *BCL2* and *MYC* translocations, of whom only one patient developed CNS relapse (FISH data were not included in the statistical analysis due to the low number of CNS relapses within the double-hit DLBCL).

CNS-IPI and COO were combined (1 point for high CNS-IPI, 1 point for ABC or unclassified COO) to create a modified risk stratification model, CNS-IPI-C. Three CNS-IPI-C subgroups were identified as having low (no risk factor, n = 450 [48.2%]), intermediate (1 risk factor, n = 408 [43.7%]), and high (2 risk factors, n = 75 [8.0%]) CNS relapse risk. The 2-year CNS relapse rates were 0.5% (95% CI, 0.0–1.3), 4.4% (95% CI, 2.2–6.6), and 15.2% (95% CI, 5.4–24.0), respectively, resulting in a 22-fold

higher risk of CNS relapse in the high- vs low-risk groups (Figure 2; Supplemental Table S3).

Mutational profile

Mutational profiles were available in 499 of 1,418 patients (12 of 38 patients with CNS relapse; 487 of 1,380 without CNS relapse; Supplemental Table S2). A detailed description of all gene alterations for the patients with CNS relapse is listed in Supplemental Table S4. *CDKN2A* was the most frequently (8 of 12; 66.6%) altered gene in patients who developed CNS relapse, with seven cases having homozygous deletion of *CDKN2A* and one case harboring nonsynonymous *CDKN2A* mutation; in the population of patients without CNS relapse, the prevalence *of CDKN2A* gene alterations was 21.6% (105 of 487). On multivariate analysis, *CDKN2A* gene alterations were associated with higher risk of CNS relapse (HR, 7.2; 95% CI, 2.1–25.0; P = .002) independent of clinical factors. The impact of *CDKN2A* gene alterations on CNS relapse risk was weakened after inclusion of COO into the model (HR, 3.6; 95% CI, 0.93–14.0; P = .064). Alterations of genes known to deregulate NFκB signaling were also observed, such as mutations of *MYD88*, which were found in 5 (42%) of 12 cases compared with 78 (16.0%) of 487 cases in the cohort with no CNS relapse. Three of the 5 patients with *MYD88* mutation had simultaneous *CD79B* mutation.

Discussion

The current analysis of GOYA evaluated risk factors associated with CNS relapse in newly diagnosed DLBCL patients treated with anti-CD20-based immunochemotherapy (R- or G-CHOP). We found no difference in CNS relapse risk

between R and G, with the incidence of CNS relapse similar in both treatment arms and consistent with the literature.[1]

With these data, we have provided an independent validation of the CNS-IPI prognostic model.[1] Patients with high CNS-IPI scores had significantly higher risk of CNS relapse than those with intermediate or low CNS-IPI scores. High CNS-IPI score was also an independent risk factor for CNS relapse on multivariate analysis. The 2-year CNS relapse rate for the high-risk CNS-IPI subgroup in GOYA (8.9%) was consistent with previous data from Schmitz and colleagues (10.2%).[1] No significant difference in the incidence of CNS relapse was observed between the intermediate- and low-risk CNS-IPI groups. This may be due to differences in baseline patient characteristics in the DSHNHL/MInT (testing cohort for CNS-IPI building) and GOYA study cohorts.[1] In the current study, we confirmed that CNS-IPI is a valuable clinical tool for identification of DLBCL patients with high CNS relapse risk.

Most primary DLBCLs of the CNS resemble the ABC subtype, suggesting that this biological subtype may be prone to CNS infiltration.[21] In the current study, patients with ABC and unclassified DLBCL had significantly higher CNS relapse risk compared with GCB, and in the multivariate analysis, COO and a high CNS-IPI score were shown to be independent risk factors for CNS relapse. Previous data by Savage and colleagues showed that BCL2/MYC dual-expression is associated with higher probability of CNS relapse.[9] Given the association of the ABC subtype with dual-expression of BCL2 and MYC proteins, we analyzed whether the higher risk of CNS relapse, at least in patients with ABC DLBCL, is related to the high prevalence of BCL2/MYC dual-expression. Surprisingly, we did not observe a higher incidence of CNS relapse in BCL2/MYC dual-expressers compared with non-dual-expressers

in univariate or multivariate analyses, which may be due to the higher prevalence of BCL2/MYC dual-expression (driven by a high rate of MYC positivity) in the GOYA study compared with the population in Savage and colleagues (42.1% vs 29.7%, respectively).[9,23] The reason for the high rate of MYC positivity detected in the GOYA study is not entirely clear. One possible explanation is that the proportion of patients enrolled with low IPI scores (or low CNS-IPI) was relatively low, and there was therefore a high proportion of high-risk patients who are more likely to be BCL2/MYC dual expressers. Larger studies may provide further insight.

Primary CNS lymphomas frequently, if not uniformly, exhibit biallelic loss of *CDKN2A*, resulting in cell cycle and p53 pathway deregulation, or mutations of *MYD88* and *CD79B*, thereby deregulating NFκB and B-cell receptor signaling.[21,28-31] Although data on the mutational profile were only available for a limited number of patients, *CDKN2A* loss and mutation of *MYD88* were the most commonly observed alterations in patients with CNS relapse. In the multivariate analysis, *CDKN2A* loss was associated with higher risk of CNS relapse independent of clinical factors. However, the impact of *CDKN2A* loss on the risk of CNS relapse was weaker in a model that included COO, probably due to the association of *CDKN2A* alterations with the ABC subtype, which has been demonstrated in GOYA as well as other studies.[32,33] Due to the limited number of patients with CNS relapse and mutational profile data available in the GOYA study, further studies are needed to confirm our hypothesis and to explore the impact of specific gene alterations on the risk of CNS relapse, especially in the context of particular COO subtypes.

Because ABC/unclassified COO subtypes and high CNS-IPI were independent risk factors for CNS relapse, we combined both factors to improve the risk stratification ability of CNS-IPI, resulting in a modified CNS-IPI-C model. CNS-IPI-C allowed the

identification of three subgroups with different 2-year CNS relapse risks. This incorporation of biomarkers into the CNS-IPI-C model improved the discrimination of subgroups with a very low and high 2-year CNS relapse risk compared with the CNS-IPI model (2-year relapse rate in low- and high-risk subgroups 0.5% vs 1.4% and 15.2% vs 9.6%, respectively). This could help identify patients who should undergo a more comprehensive examination of the CNS to exclude asymptomatic CNS lymphoma involvement. It may also identify patients who could potentially benefit from treatment with effective prophylaxis to reduce CNS relapse risk.[34,35] Last but not least, CNS-IPI-C identifies a large subgroup of patients with a very low risk of CNS relapse who could be spared invasive diagnostic and prophylactic interventions. However, it must be noted that CNS-IPI-C needs to be validated in an independent cohort of DLBCL patients before its potential clinical use.

There is growing evidence that CNS prophylaxis with intrathecal methotrexate is not sufficient to prevent CNS relapse.[5,36] Some trials indicate that intravenous high-dose methotrexate (3 g/m^2) can prevent CNS relapse;[37] however, treatment can be associated with significant toxicity, and an acceptable risk-benefit ratio should be carefully considered. Overall, 9.9% of patients were treated with prophylactic intrathecal chemotherapy in GOYA. We did not observe a significant difference in the incidence of CNS relapse in patients who received intrathecal chemotherapy compared with those who did not, neither in the whole cohort nor in the different risk groups according to CNS-IPI. It must be noted, however, that GOYA was not designed to assess the impact of CNS prophylaxis on CNS relapse risk. CNS prophylaxis was indicated and administered upon investigator decision, based on institutional practice, resulting in heterogeneous schedules and doses. Randomized clinical trials would be required to define appropriate CNS prophylaxis in DLBCL.

In conclusion, using the largest prospective dataset of previously untreated DLBCL with relevant biomarker data to date, we validated the CNS-IPI clinical prognostic model and demonstrated that ABC and unclassified DLBCL are associated with higher CNS relapse risk compared with GCB DLBCL. Combining CNS-IPI and COO helped to improve stratification of DLBCL patients with different CNS relapse risks.

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Role of the funding source

The funder was involved in trial design, administration or conduct of study procedures, coordination of data collection, and data analysis and interpretation. Corresponding authors (M.K. and M.T.) and Roche authors (E.A.P., E.S.G., C.B., M.Z.O., G.F.R., and T.N.) had access to all data in the study; all other authors had access to the final study report. MK had final responsibility for the decision to submit for publication.

Authorship

M.K., M.Z.O., G.R.F.R., T.N., and M.T. took part in conception and design of the analysis. L.H.S., I.B.B., F.C., J.J., M.M., D.S., U.V., F.Z., Q.Z., and M.T. provided study materials or patients. All authors contributed to analysing and interpreting the data, writing the article, and provided final approval of the manuscript.

Conflicts of interest

M.K. reports employment with F. Hoffmann-La Roche Ltd during the time of the analysis. L.H.S. reports consultancy and honoraria from Roche/Genentech, Amgen, Janssen, Celgene, AbbVie, and Seattle Genetics. I.B.B. reports advisory board

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TABLES

Table 1. Key baseline clinical characteristics (CNS-IPI risk factors, CNS-IPI score) of patients who developed CNS relapse compared with patients with no CNS relapse and the overall GOYA study population

Characteristic	CNS relapse (n = 38)	No CNS relapse (n = 1,380)	All patients (N = 1,418)
Median age (range), years	**66.5 (21-81)**	**61.0 (18-86)**	62.0 (18-86)
<60	13 (34.2)	591 (42.8)	604 (42.6)
≥60	25 (65.8)	789 (57.2)	814 (57.4)
ECOG PS			
0-1	31 (81.6)	1,200 (87.0)	1,231 (86.9)
2-3	7 (18.4)	179 (13.0)	186 (13.1)
Ann Arbor Stage			
I and II	**4 (10.5)**	**337 (24.4)**	341 (24.1)
III and IV	**34 (89.5)**	**1,042 (75.6)**	1,076 (75.9)
Elevated LDH	**26 (68.4)**	**790 (57.5)**	816 (57.7)
Number of extranodal sites			
0-1	**15 (39.5)**	**900 (65.2)**	915 (64.5)
>1	**23 (60.5)**	**480 (34.8)**	503 (35.5)
Involvement of kidneys and/or adrenal glands	**11 (28.9)**	**80 (5.8)**	91 (6.4)
CNS-IPI			
Low (0-1)	4 (10.5)	275 (20.0)	279 (19.7)
Intermediate (2-3)	**16 (42.1)**	**878 (63.6)**	894 (63.0)
High (4-6)	**18 (47.4)**	**227 (16.5)**	245 (17.3)

NOTE. Data are presented as No. (%) unless otherwise noted. Data for ECOG PS and Ann Arbor Stage were not available in one case, and data on LDH were not available in five cases. Differences ≥ 10% between CNS relapse/no relapse groups are highlighted in bold. CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

Table 2. Key clinical and biomarker characteristics of patients with distinct COO subtypes: GCB, Unclassified, and ABC DLBCL

Characteristic	GCB (n = 540)	Unclassified (n = 150)	ABC (n = 243)
Median age (range), years	62.5 (18-83)	62.0 (21-83)	64.0 (29-86)
<60	228 (42.2)	59 (39.3)	70 (28.8)
≥60	312 (57.8)	91 (60.7)	173 (71.2)
ECOG PS			
0-1	475 (88.1)	126 (84.0)	209 (86.0)
2-3	64 (11.9)	24 (16.0)	34 (14.0)
Ann Arbor Stage			
I and II	146 (27.0)	34 (22.7)	52 (21.4)
III and IV	394 (73.0)	116 (77.3)	191 (78.6)
Elevated LDH	308 (57.1)	76 (50.7)	169 (70.4)
Number of extranodal sites			
0-1	355 (65.7)	95 (63.3)	158 (65.0)
>1	185 (34.3)	55 (36.7)	85 (35.0)
Involvement of kidneys and/or adrenal glands	36 (6.7)	9 (6.0)	13 (5.3)
CNS-IPI			
Low (0-1)	115 (21.3)	29 (19.3)	28 (11.5)
Intermediate (2-3)	335 (62.0)	97 (64.7)	164 (67.5)
High (4-6)	90 (16.7)	24 (16.0)	51 (21.0)
BCL2/MYC dual-expression	n = 381	n = 114	n = 193
Non-dual expressers	264 (69.3)	72 (63.2)	57 (29.5)
Dual expressers	117 (30.7)	42 (36.8)	136 (70.5)

NOTE. Data are presented as No. (%) unless otherwise noted. Data for ECOG PS were not available in one case, and data on LDH were not available in three cases.
ABC, activated B-cell–like; CNS, central nervous system; COO, cell-of-origin; DLBCL, diffuse large B-cell lymphoma; ECOG PS, Eastern Cooperative Oncology Group performance status; GCB, germinal center B-cell–like; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

Table 3. Results of multivariate Cox regression analysis on factors associated with CNS relapse in the COO-available population (n = 933), CNS relapses (n = 30)

Factor	HR*	95% CI	*P* value
CNS-IPI intermediate (*v* low)	0.88	0.29-2.74	.8312
CNS-IPI high (*v* low)	**3.97**	**1.28–12.33**	**.0172**
ABC COO (*v* GCB)	**5.18**	**2.09–12.87**	**.0004**
Unclassified COO (*v* GCB)	**4.18**	**1.50–11.66**	**.0062**

*Adjusted for study randomization stratification factors (number of planned chemotherapy cycles, geographic region).
ABC, activated B-cell–like; CNS, central nervous system; COO, cell-of-origin; GCB, germinal center B-cell–like; HR, hazard ratio; IPI, International Prognostic Index.

Table 4. Results of multivariate Cox regression analysis on factors associated with CNS relapse in the COO and BCL2/MYC dual-expression status-available population (n = 688), CNS relapses (n = 22)

Factor	HR*	95% CI	*P* value
CNS-IPI intermediate (*v* low)	0.75	0.23–2.45	.6378
CNS-IPI high (*v* low)	2.76	0.81–9.42	.1042
ABC COO (*v* GCB)	**4.78**	**1.49–15.29**	**.0084**
Unclassified COO (*v* GCB)	**4.24**	**1.32–13.61**	**.0151**
BCL2/MYC dual expresser (*v* non-dual expresser)	0.83	0.34–2.06	.6931

*Adjusted for study randomization stratification factors (number of planned chemotherapy cycles, geographic region).
ABC, activated B-cell–like; CNS, central nervous system; COO, cell-of-origin; GCB, germinal center B-cell–like; HR, hazard ratio; IPI, International Prognostic Index.

FIGURE LEGENDS

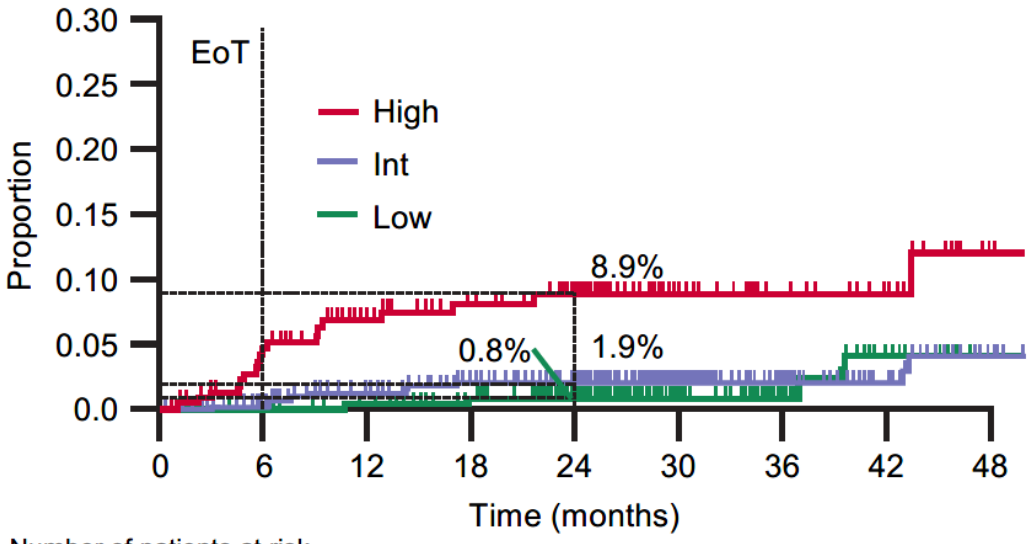
Figure 1. Risk of CNS relapse by CNS-IPI categories in (A) overall GOYA study population (N = 1,418), and (B) COO available population (n = 933). CNS, central nervous system; COO, cell-of-origin; EoT, end of treatment; IPI, International Prognostic Index.

Figure 2. Risk of CNS relapse by CNS-IPI and COO (CNS-IPI-C) in the COO available population (n = 933). ABC, activated B-cell–like; CNS, central nervous system; COO, cell-of-origin; EoT, end of treatment; H-R, high risk; IPI, International Prognostic Index; I-R, intermediate risk; L-R, low risk; UNCL, unclassified.

FIGURES

Figure 1

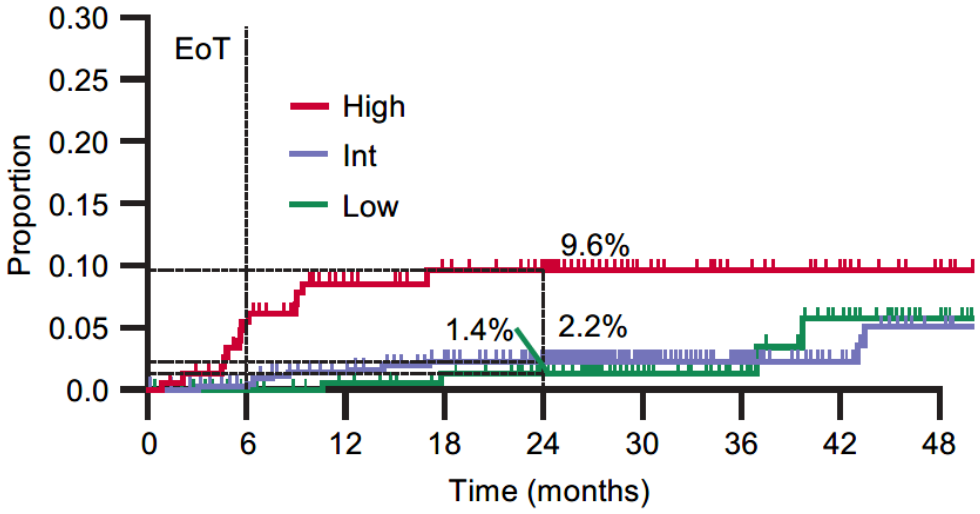
A



Number of patients at risk

High	245	192	150	132	113	65	45	35	14
Int	894	783	668	623	529	299	186	118	48
Low	279	263	249	235	196	104	69	46	18

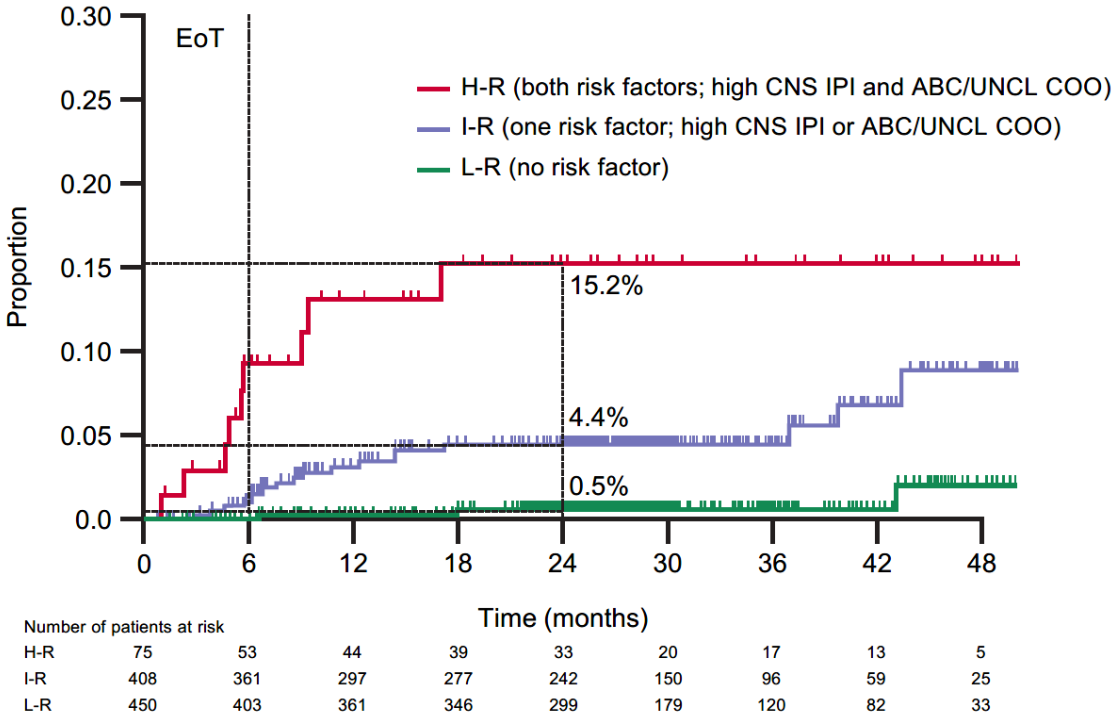
B



Number of patients at risk

High	165	130	100	91	78	48	36	28	13
Int	596	527	454	430	374	223	145	90	38
Low	172	160	148	141	122	78	52	36	12

Figure 2


EoT
H-R (both risk factors; high CNS IPI and ABC/UNCL COO)
I-R (one risk factor; high CNS IPI or ABC/UNCL COO)
L-R (no risk factor)
Proportion
0.30
0.25
0.20
0.15
0.10
0.05
0.0
15.2%
4.4%
0.5%
0
6
12
18
24
30
36
42
48
Time (months)
Number of patients at risk
H-R 75 53 44 39 33 20 17 13 5
I-R 408 361 297 277 242 150 96 59 25
L-R 450 403 361 346 299 179 120 82 33