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CLINICAL TRIALS AND OBSERVATIONS

*Comment on Morschhauser et al, page 600*

# Bcl-2 inhibition in DLBCL: "the times they are a-changing"?

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**In this issue of *Blood*, Morschhauser et al report the results of the phase 2 CAVALLI study in 206 patients with International Prognostic Index (IPI) 2-5 diffuse large B-cell lymphoma (DLBCL).[1] The authors demonstrate that the addition of venetoclax (a selective and potent B-cell lymphoma-2 [Bcl-2] inhibitor) to rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) has the potential to improve the outcome of patients with Bcl-2 overexpressed DLBCL despite a higher incidence of adverse effects.**

This study is particularly exciting because vaulting over the wall of R-CHOP results in DLBCL has been the dream of many researchers for years. The addition of rituximab improved the efficacy of the CHOP regimen in DLBCL with 55% to 60% of patients cured and is still the standard of care.[2] However, treatment fails for 40% to 45% of patients, and this prompted many attempts to improve the efficacy of first-line treatment for DLBCL. Chemotherapy regimens were modified to use infusional, dose-adjusted drugs or consolidation after R-CHOP with high-dose chemotherapy and autologous stem cell transplantation. Despite promising results in phase 2 studies, randomized phase 3 trials failed to show any overall survival benefit over R-CHOP.[3,4]

The substitution of rituximab with obinutuzumab was assessed in the international, randomized phase 3 GOYA-BO21005 (hereafter GOYA) study in 1418 DLBCL patients. Although obinutuzumab was more active than rituximab in follicular lymphoma and chronic lymphocytic leukemia (CLL), this did not happen in DLBCL. The GOYA study did not show an advantage over R-CHOP.[5] Nevertheless, the GOYA study has been considered a benchmark for other DLBCL studies, and indeed Morschhauser et al compare their results with those from the GOYA study.

Overall, these data emphasize that chemoimmunotherapy resistance cannot be easily overcome simply by modifying chemotherapy regimens or by substituting a new anti-CD20 antibody. This may be because of the heterogeneity of DLBCL, which has 3 major biologically distinct pathophysiologic entities based on cell-of-origin (COO): germinal center B-cell–like (GCB), activated B-cell–like (ABC), and unclassified, with the ABC subgroup having the worst prognosis when treated with R-CHOP.[6] These data prompted a change in strategy for treating DLBCL and a shift to targeting the group with the worst prognosis (ie, the ABC group) with appropriate drugs. Two drugs, ibrutinib and lenalidomide, showed activity in non-GCB/ABC DLBCL subgroups in early phase 1/2 studies with positive clinical results. Randomized phase 3 studies tested the addition of ibrutinib or lenalidomide to R-CHOP vs placebo/R-CHOP (PHOENIX and ROBUST studies) in non-GCB/ABC IPI 2-5 DLBCL. Unfortunately, there were no differences in 2-year progression-free survival (PFS) rates between the arms.[7,8]

The additional biological complexity of DLBCL explains these results. Indeed, a recent molecular classification of DLBCL identified 5 distinct molecular subsets and reported wider heterogeneity within the COO subgroup whose patients had very different prognoses.[9] However, further subgroup analyses of the PHOENIX and ROBUST studies suggested some benefits in patients with specific clinical or biological characteristics. Patients who were young and/or those with overexpression of Bcl-2 and Myc had a more favorable outcome with ibrutinib–R-CHOP in the PHOENIX trial, and clinically high-risk patients (ie, patients with IPI 3-5) had a more favorable outcome with lenalidomide–R-CHOP in the Robust study. These data open a window to a possible successful strategy to improve R-CHOP results if patients are better selected for new treatments.

The Morschhauser et al study addressed a specific DLBCL subgroup with poor prognosis: patients with overexpressed Bcl-2. Indeed, overexpression of Bcl-2 (an anti-apoptotic protein) identifies DLBCL patients with inferior outcomes who are resistant to R-CHOP. Moreover, patients with concurrent overexpression of Bcl-2 and Myc proteins (known as double expressors [DELs]) have a poor prognosis when treated with R-CHOP. Overall, these patients represent 25% to 45% of all cases of DLBCL. Moreover, data from GOYA and other studies suggest that Bcl-2 overexpression is the main driver of poor prognosis in DELs, and counteracting Bcl-2 may be a way to improve their outcome.

Morschhauser et al targeted these poor-prognosis subgroups. The authors added venetoclax to R-CHOP in 206 patients with advanced-stage DLBCL. That study was nonrandomized, but the authors made

**First-line chemoimmunotherapy combinations for newly diagnosed DLBCL**

| Scheme (trial) | Study phase | No. of patients | Median age (range) | Biological characteristics | ORR/ CR (%) | PFS/EFS (%) at time period | OS (%) at time period | Primary end point | HR |
|---|---|---|---|---|---|---|---|---|---|
| Venetoclax + R-CHOP (CAVALLI)[1] | II | 206 | 65 (18-85) | All DLBCL | 83/69 | 80 (2 y) | 86 (2 y) | CR | — |
| Venetoclax + R-CHOP Bcl-2-positive patients (CAVALLI)[1] | II | 104 | NA | All DLBCL | 83/64 | 78 (2 y) | 83 (2 y) | CR | — |
| Rituximab-CHOP[2] | III | 202* | 69 (60-80) | All DLBCL | 82/52 | 57 (2 y) | 70 (2 y) | EFS | 0.58 |
| DA-EPOCH-R[3] | III | 241* | 58 (19-84) | All DLBCL | 87/58 | 80 (2 y) | 86 (2 y) | PFS | 0.93 |
| HDC + ASCT[4] | III | 199* | 48 (36-56) | All DLBCL | 79/76 | 70 (5 y) | 78 (5 y) | EFS | 0.65 |
| G-CHOP (GOYA)[5] | III | 704* | 62 (18-86) | All DLBCL | 77/57 | 64 (5 y) | 77 (5 y) | PFS | 0.94 |
| Ibrutinib + R-CHOP (PHOENIX)[7] | III | 419* | 63 (19-88) | Non-GCB | 89/67 | 70 (2 y)† | 85 (2 y)† | EFS | 0.93 |
| R2-CHOP (ROBUST)[8] | III | 285* | 65 (21-82) | ABC | 91/69 | 75 (2 y)† | 79 (2 y) | PFS | 0.85 |
| RLI + R-CHOP‡ (SMART START)[10] | Single-arm II | 60 | 63 (29-83) | Non-GCB | 100/95 | 92 (1 y) | 96 (1 y) | ORR/CR | — |

CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; DA-EPOCH-R, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab; EFS, event-free survival; G, obinutuzumab; HDC + ASCT, high-dose chemotherapy plus autologous stem cell transplantation; HR, hazard ratio; NA, not available; ORR, overall response rate; OS, overall survival; R, rituximab; R2, rituximab plus lenalidomide; RLI, rituximab, lenalidomide, and ibrutinib.

*Considering experimental arm only.

†Approximate data obtained from reported Kaplan-Meier curves.

‡CHOP or dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) per treating physician choice.

an effort to compare the results with matched R-CHOP controls from the GOYA study (564 patients). Positron emission tomography complete response (PET-CR) rates, the primary end point, were superimposable on those of the GOYA cohort (69% and 63%). Interestingly, the addition of venetoclax was able to significantly increase 2-year PFS rate (the secondary end point) compared with GOYA results, namely in Bcl-2–positive patients (78% vs 62%; hazard ratio, 0.55; 95% confidence interval, 0.34-0.89), suggesting that the addition of venetoclax may induce a much deeper response, as occurred in CLL, leading to a reduction in the rate of progression (see table).

Although treatment was manageable, grade 3 to 4 adverse event rates were higher in CAVALLI compared with GOYA for neutropenia (68% vs 39%) and infections (23% vs 16%), which shows the need for more stringent, well-defined prophylactic measures when a novel drug is added to R-CHOP, a policy that was not clearly adopted in the Morschhauser et al study. However, the costs of adverse effects of treatment, if manageable, are worth paying when they are balanced with a long-term benefit, as happened in the Morschhauser et al study.

Limitations of this study include the nonrandomized design, the lack of PET-CR differences (ie, the primary end point) compared with the GOYA study, and the unbalanced comparison between the CAVALLI and GOYA studies, the first conducted in 50 sites and the second in 207 sites in a larger worldwide setting with different level of expertise. Nevertheless, the difference in PFS for Bcl-2–positive patients is wide enough and is also a good signal for conducting further studies with this combination.

The emerging molecular complexity of DLBCL could lead to novel therapeutic strategies with the identification of different molecular pathways targeted by appropriate drugs, possibly added in multiple combinations to chemoimmunotherapy to overcome refractoriness to chemotherapy.[9] An intriguing example is the SMART-START study that combines rituximab, lenalidomide, and ibrutinib as a chemotherapy-free approach followed by CHOP or etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) in 60 patients with non-GCB DLBCL. The preliminary 92% rate for PET-CR is encouraging and merits further exploration.[10]

"The times they are a-changin'" as Bob Dylan used to sing, may also be true for the treatment of at least a subset of DLBCL patients, but the data presented here underline that our work still has a long way to go.

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**IMMUNOBIOLOGY AND IMMUNOTHERAPY**

*Comment on Daher et al, page 624*

# Releasing the brake in CAR natural killer cells

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**In this issue of *Blood*, Daher et al[1] present a strategy to improve natural killer (NK) cell effector function that combines chimeric antigen receptor (CAR) engineering and gene editing of a cytokine-related immune checkpoint. Their study provides preclinical data on the mechanistic synergy of these 2 approaches and might have an impact on the future development of NK cell–based cancer immunotherapy.**

Recent advances in cancer immunotherapy have mainly focused on engineering T cells to express a CAR against specific tumor antigens. Although significant clinical efficacy has been achieved in B-cell malignancies, the application of the existing CAR T-cell products approved by the US Food and Drug Administration still faces logistic and clinical challenges, such as timely collection and expansion of sufficient numbers of autologous gene–modified T cells in heavily pretreated patients. NK cells are attractive alternative candidates for novel approaches in cancer immunotherapy because they mediate potent cytotoxicity against tumor cells and can potentially be used as readily available off-the-shelf products.[2]

Liu et al have previously demonstrated that cord blood–derived NK cells engineered to secrete interleukin-15 (IL-15) and to express a CD19-targeted CAR exhibit potent antitumor activity and long-term persistence.[3] In the Daher et al study, the authors investigate the question of whether the effector function and persistence of these IL-15–secreting, so-called "armored" CD19 CAR-NK cells can be further improved by deleting cytokine-inducible Src homology 2-containing protein (CIS), a key negative regulator of IL-15 signaling.

CIS, which is encoded by the *CISH* gene, is a member of the suppressor of cytokine signaling (SOCS) family of proteins. CIS is induced by cytokines such as IL-15 and IL-2 and acts as an important intracellular immune checkpoint in NK cells.[4] Specifically, it uses a negative feedback loop to suppress signaling of the pleiotropic cytokine IL-15, which is known to drive NK-cell activation, expansion, and persistence.[4]

By using a series of in vitro and in vivo experiments, the authors provide a detailed characterization of CD19 CAR-NK cells undergoing CRISPR/Cas9-mediated *CISH* knockout (KO). On a phenotypic level, *CISH* deletion was shown to be associated with prominent features of activation, proliferation, and cytotoxicity. Interestingly, despite the activation phenotype caused by *CISH* KO, no evidence of activation-induced cell death was observed after stimulation with tumor targets in vitro. The effects of *CISH* deletion on the transcriptome included upregulation of genes related to inflammatory and immune responses as well as cytokine signaling. Notably, pathways involved in signaling of interferon-γ, tumor necrosis factor-α, IL-2/STAT5 and IL-6/JAK/STAT3 were enhanced.

Taken together, these results support the hypothesis that targeting CIS in CAR-NK cells removes a critical immune checkpoint. When tested against $CD19^+$ Raji lymphoma cells in vitro, *CISH* KO CAR-NK cells displayed greater cytotoxicity against their targets. From a metabolic standpoint, the study provides evidence that this gain of effector function can be attributed to enhanced IL-15 signaling secondary to removal of the CIS checkpoint, which leads to activation of the AKT/mTOR pathway and c-Myc pathway (see figure). The authors suggest that endogenously secreted IL-15 by CAR-NK cells has to overcome a lower threshold of activity to trigger the AKT/mTOR pathway, which is known to be involved