

## Genetics and diffuse large B-Cell lymphoma

RABIN NIROULA, MD; JAMES BUTERA, MD

### ABSTRACT

Diffuse large B-Cell lymphoma (DLBCL) is one of the most common and aggressive subtypes of non-Hodgkin's lymphoma (NHL). Gene expression profiling (GEP) studies have identified at least two distinct molecular subtypes of DLBCL termed as germinal center B-cell (GCB) and activated B-cell (ABC). These molecular subtypes represent lymphomas that are driven by very different intracellular oncogenic signaling pathways which have prognostic value and could potentially be exploited for therapeutic benefit in future. There are other oncogenes, namely BCL-2, BCL-6 and MYC, which have been associated with the pathogenesis of DLBCL. Concurrent presence of two oncogenes is present in about 5% of DLBCL and it is termed "double hit lymphoma" (DHL). DHL are associated with an aggressive clinical course and do not respond well to the standard DLBCL immune-chemotherapy regimen, RCHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone). Other aggressive therapeutic approaches including autologous bone marrow transplant have not shown any survival benefit in this subgroup of DLBCL patients. New strategies in development to address this resistance in DHL include the regimen DA-EPOCH-R (dose adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab). Recent studies have shown increased sensitivity of DHL to DA-EPOCH-R chemotherapy and will likely be the new standard of care in this subset of DLBCL patients in the future.

**KEYWORDS:** Diffuse large B cell lymphoma, Activated B-Cell, Germinal center B-Cell, MYC, BCL-6 and BCL-2 oncogenes, RCHOP, DA-EPOCH-R.

Worldwide, Diffuse large B cell lymphoma represents the most common subtype of non-Hodgkin's lymphoma (NHL) and it accounts for 30–40% of the newly diagnosed NHL cases.<sup>1</sup> DLBCL typically behaves aggressively, quickly evolving over weeks to months and can be fatal without treatment. However, with therapy DLBCL is curable in more than 60% of patients that are treated with combination immuno-chemotherapy, of which the most common regimen employed is RCHOP.<sup>2</sup> Prognosis of DLBCL has traditionally been based on five patient characteristics: age, performance

status, number of extra nodal sites of lymphoma involvement, stage and LDH value. Until recently, little was known regarding the impact of the molecular profile of malignant lymphoma cells on the treatment strategy and prognosis of patients with DLBCL. However, recent discoveries in the molecular biology of DLBCL have resulted in the identification of a subset of DLBCL which does poorly with standard RCHOP therapy.

Gene expression profiling (GEP) studies in DLBCL have identified at least 2 distinct molecular subtypes, germinal center B cell (GCB) and activated B cell (ABC).<sup>3</sup> These molecular subtypes are believed to represent lymphomas arising from different stages of lymphoid differentiation. The GCB subtype arises from the centroblasts, whereas the ABC subtype arises from the plasmablastic cell just prior to germinal center exit.<sup>4</sup> These molecular subtypes not only represent different intracellular oncogenic signaling pathways but are a result of the oncogenic pathway perturbations that result due to recurrent mutations, gains and losses of genetic material, and characteristic translocations. This molecular distinction has prognostic implications; the ABC subtype has an inferior outcome following R-CHOP chemotherapy compared to GCB subtype. One study has reported a 3-year progression-free survival (PFS) of 40% in ABC group vs. 75% in GCB group.<sup>5</sup>

The major oncogenic pathways that have been identified with varying frequencies in GCB DLBCL are c-rel amplification, EZH2 (histone methyl transferase) mutation, deletion of PTEN (causes activation of phosphatidylinositol 3 kinase/AKT/mTOR signaling pathway which are instrumental in cellular growth and metabolism).<sup>6</sup> About 30% of GCB also have t<sup>14,18</sup> which causes increased expression of anti-apoptotic protein Bcl2.

In contrast, the pathogenic hallmark of ABC DLBCL is the constitutive activation of the NF- $\kappa$ B signaling pathway, which promotes cell survival, proliferation and inhibition of apoptosis. The activation of the NF- $\kappa$ B pathway is largely due to constitutive activation of the CBM signaling complex (formed by CARD11, BCL10 and MALT1). The CBM complex can be activated by different genetic aberrations; 10% harbor activating mutations of CARD11 and in the remaining cases chronic active B-cell receptor signaling engages the CBM pathway. Chronic active B-cell receptor signaling is mediated through mutations in some B-cell receptor (CD79A or CD79B) and downstream kinases namely spleen tyrosine

kinase (SYK), Phosphatidylinositol3kinase (PI3K), bruton tyrosine kinase (BTK) and protein kinase C b (PKCb).<sup>7</sup> Other mutations that have been observed in varying frequencies are mutations in MYD88, loss of TNFAIP3 which result in up-regulation and loss of inhibition respectively of NF-kB and Janus kinase pathways.<sup>8</sup>

Along with the identification of intracellular oncogenic pathways, GEP studies have also identified molecular signatures related to the microenvironment of the tumor cells that are independent of these molecular subtypes. Stromal-1 signature reflects extracellular matrix deposition and infiltration of the tumor by macrophages and stromal-2 signature identifies tumors associated with high level of angiogenesis and high density of blood vessels. These molecular subtypes have been correlated with outcome; stromal 1 represents a prognostically favorable group compared to stromal 2 subtype.<sup>5</sup> In addition to these, recurring lesions in the genes involved in immune recognition and antigen presenting functions have been recognized, suggesting that escape from immune surveillance plays an important role in the pathogenesis of DLBCL.<sup>9</sup> There are, however, a subset of DLBCL whose biology cannot be explained by genomic events and transcriptional programs that are identified on the GEP, suggesting an additional layer of regulation. Recently somatic mutations in the epigenetic machinery have been identified suggesting the significance of epigenetic regulation in the normal B cell development and in lymphomagenesis. These epigenetic subgroups of DLBCL reflect the variability in DNA methylation, which has also been associated with clinical outcome.<sup>10</sup>

The growing understanding of the molecular pathways in DLBCL has provided an opportunity to pharmacologically target these pathways to improve clinical outcomes in DLBCL. However, further work is needed to translate the recent discoveries to the clinical setting; this can be done by clinical trials, which support the use of tumor genetics in the formulation of therapeutic plans. There are many ongoing trials which are looking at agents that target various molecules in the oncogenic pathways of DLBCL. Immunomodulatory agents which target the NFkB pathways like bortezomib and lenalidomide have been looked in non GCB DLBCL in retrospective studies. One study showed a higher response rate of 83% vs. 13% (p<0.001) and overall survival of 10.8 vs. 3.4 months (p=0.003) with the addition of bortezomib to chemotherapy in relapsed/refractory DLBCL.<sup>11</sup> Similarly, studies using lenalidomide have demonstrated a high response rate in relapsed/refractory setting. Numerous phase III trials are ongoing which are looking at addition of these immunomodulatory agents with standard chemotherapy in non GCB DLBCL in first line setting.

One of the better-understood aspects of the pathogenesis in DLBCL is the alteration in the oncogenes and tumor suppression genes. Three such oncogenes are c MYC, BCL-2 and BCL-6, key regulation of cellular proliferation(c MYC) and apoptosis (BCL-2 and BCL-6). BCL-2 and BCL-6

translocations are present in about 15 and 29% of DLBCL respectively.<sup>12,13</sup> The c-MYC translocation is present in about 5-10% of DLBCL.<sup>14</sup> Isolated presence of BCL-2 genetic aberration does not have independent prognostic value. Furthermore, it is controversial whether the c-MYC translocation alone has prognostic value in patients with DLBCL. However, recent studies have revealed that the impact of MYC is strongly influenced by BCL-2 or BCL-6. The presence of concurrent MYC and BCL-2 or BCL-6 translocation in patients with DLBCL, also known as "double hit lymphoma" (DHL), which has been associated with a very aggressive clinical course and an overall worse survival with standard R-CHOP chemotherapy. DHL occurs in nearly 5% of cases of DLBCL.<sup>15</sup>

Numerous studies have correlated the presence of MYC rearrangement and BCL-2 or BCL-6 with a poorer outcome in DLBCL treated with standard chemotherapy RCHOP.<sup>15,16</sup> One example is a retrospective study by Akyurek et al investigating the impact of c-MYC, BCL-2 and BCL-6 rearrangements in 239 patients with DLBCL treated with RCHOP therapy. In patients with DHL, outcome was extremely poor with a median survival of 9 months and a 2-year overall survival (OS) rate of only 14% vs. 78% for non-DHL patients (Progression free survival, p = 0.003 and OS, p < 0.001).<sup>17</sup> Given the extremely poor outcome seen for this subset of DLBCL patients, clearly newer therapies are needed for DHL patients.

However, even though the double hit lymphoma have unacceptably poor cure rate with standard therapy, the optimal management approaches in this population of patients remains to be defined. As this subtype of lymphomas is rare, there are no large prospective studies evaluating the role of alternative forms of therapy in this poor prognosis group. One approach has been to use more aggressive chemotherapy treatments strategies, such as chemotherapy regimens used to treat the more aggressive form of NHL, Burkitt lymphoma, which consist of higher doses and more intensive chemotherapy cycles. However, one limiting factor is the high median age of patients with DLBCL, who due to co-morbidities may not be able to tolerate more aggressive regimens. One such attempt at this approach is the use of the aggressive chemotherapy regimen R-Hyper-CVAD (rituximab, fractionated cyclophosphamide, adriamycin, dexamethasone alternating with high dose methotrexate, cytarabine and vincristine) frequently used to treat Burkitt lymphoma. However, the recent experience with this regimen in patients with DHL is not promising. For example, Li et al reported on a retrospective series of 52 patients with DHL treated with R-Hyper-CVAD regimen and found median OS in these patients was only 18.6 months.<sup>18</sup> Other studies have reported similar poor outcomes with R-Hyper-CVAD in DHL patients.

Another approach to improve the outcome of DHL patients is to intensify therapy with the use of autologous bone marrow transplantation (BMT). However, two retrospective

series investigating the role of autologous BMT did not demonstrate an advantage over standard immunochemotherapy regimens.<sup>19</sup>

However, one very promising approach in patients with DHL is the use of prolonged infusional chemotherapy. One such regimen is dose-adjusted EPOCH with rituximab or DA-EPOCH-R. In this regimen the chemotherapy agents are similar to RCHOP with the addition of etoposide. However, the agents vincristine, adriamycin and etoposide are given as continuous infusional therapy over a period of 4 days rather than the standard one day bolus regimen of RCHOP. Evidence for this approach comes from in vitro data that suggests that lymphoma cells of high proliferation rates may be more sensitive to a continuous exposure to chemotherapy rather than the more traditional bolus chemotherapy. Furthermore, DA-EPOCH-R has been used successfully in the more aggressive c-MYC positive Burkitt lymphoma and it has been postulated that c-MYC positive lymphoma cells may show more susceptibility to infusional regimens such as DA-EPOCH then other DLBCL cells. Moreover, DA-EPOCH-R has been shown to be well tolerated in elderly patients. One other advantage to DA-EPOCH-R is that the doses of the chemotherapy agents are adjusted up or down depending on absence or presence of associated toxicities, thereby, theoretically tailoring therapy to minimize toxicity while maximizing the doses of the chemotherapeutic agents.

One retrospective study published by Oki, et al has shown a superior rate of complete remissions (CR) with DA-EPOCH-R compared to R-CHOP in DHL patients. In this analysis, CR rates were found to be approximately 68% for DA-EPOCH-R compared to 20% for the more traditional RCHOP (p = 0.008).<sup>20</sup> Preliminary data from a recent multicenter Phase II study has shown promising activity with DA-EPOCH-R with progression free survival at 1 year of 87%.<sup>21</sup> These data appear to show patients with DHL maybe more sensitive to DA-EPOCH-R chemotherapy versus the standard RCHOP regimen. However, longer follow-up of this prospective study will help us identify if this increased sensitivity results in a survival benefit for patients with DHL treated in this manner.

## CONCLUSION

DLBCL is a very treatable and frequently curable NHL. GEP has helped us identify at least two subtypes of DLBCL (ABC and GCB) based on their different stages of lymphoid differentiation. Each of these molecular subtypes of DLBCL has different intracellular oncogenic signaling pathways. ABC subtype is associated with poor prognosis and has poor outcomes with standard chemo immunotherapy compared with GCB subtype. Along with the cells of origin, molecular signatures related to the microenvironment of the tumor cells and mutation in the epigenetic machinery have also been implicated in the prognosis of DLBCL which are independent of these molecular subtypes. Given the poor prognosis

in these subtypes of DLBCL, numerous ongoing studies are looking at agents targeting the oncogenic signaling pathways. There is another known subtype of DLBCL called double hit lymphoma which has the presence of c-MYC rearrangements in association with BCL-2 or BCL-6 rearrangements in patients with DLBCL. This delineates a population with an overall dismal prognosis for which new treatment strategies are needed. One promising mode of therapy is infusional therapy in the form of DA-EPOCH-R; however, we will need to await the results of prospective trials to ultimately determine if this regimen offers an improvement over standard approaches.

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### Authors

Rabin Niroula, MD, Roger Williams Medical Center, Department of Hematology and Oncology, Boston University School of Medicine, Providence, RI.

James Butera, MD, Clinical Associate Professor of Medicine, Department of Hematology and Oncology, Alpert Medical School of Brown University, Providence, RI.

### Correspondence

Rabin Niroula, MD  
 51 Front Street  
 Cumberland, RI 02864  
 rniroula@yahoo.com