Relevant Biomarkers for the Treatment of Hematological Malignancies

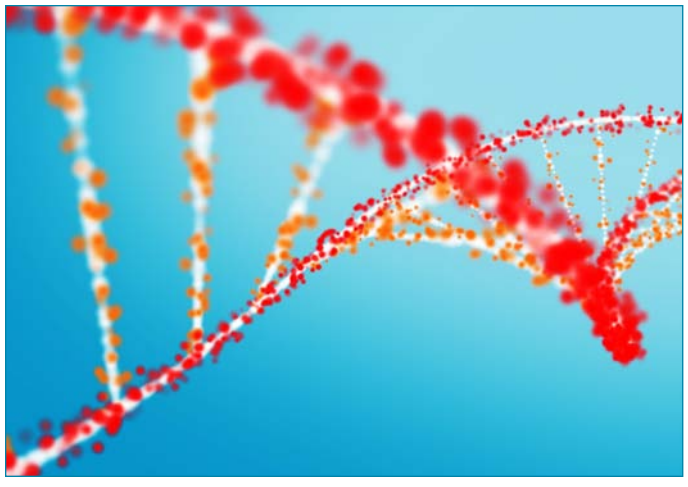
BY RICHARD SIMONEAUX

As the number of therapies increase for hematologic malignancies, there has been an increased need to find patient populations who might most benefit from these newer treatments. The demand for increased information surrounding disease characteristics has been met by the rapid development of powerful immunohistochemistry (IHC) and genetic sequencing technologies.

In one recent review, pathologist Alex Chan, MD, and colleague Ahmet Dogan, MD, PhD, both at Memorial Sloan Kettering Cancer Center, described relevant biomarkers for diffuse large B-cell lymphoma (DLBCL), one of the most frequently diagnosed non-Hodgkin lymphoma (*Surg Pathol Clinics* 2019;12(3):699-707).

DLBCL is one of the most common hematologic malignancies, comprising approximately 25-40 percent of all non-Hodgkin lymphoma cases. This disease is characterized as being genetically diverse, having a number of different presentations, pathogenic mechanisms, and therapeutic outcomes.

The use of the R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) has allowed roughly 60 percent of DLBCL patients to be cured of their disease. For the remaining approximately 40 percent, approximately half are able to attain either a partial response or complete response with currently utilized salvage chemotherapy. Consequently, the discovery of underlying disease mechanisms is paramount for treating this resistant disease.



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When queried about the heterogeneity of DLBCL, Chan replied, "The landmark gene expression profiling (GEP) study by Alizadeh (*Nature* 2000;403:503-511) found two main subtypes of DLBCL—one which showed a gene expression pattern similar to germinal center B cells (GCB subtype) and another that showed a pattern similar to activated B cells (ABC subtype). A few cases weren't classifiable by this schema, so the two subtypes (GCB and ABC) are the major subtypes we think of today."

Regarding the methods used to identify the different disease subtypes, Chan stated, "We typically use an IHC algorithm called the Hans algorithm to classify DLBCLs rather than GEP, because GEP is pretty impractical for routine clinical use. The biomarkers we examine by IHC for this algorithm are CD10, BCL6, and MUM1, but there are other algorithms which require different biomarkers as well."

DLBCL Subtype Differences

The last several years have brought dramatic improvements in the availability, costs, and speed for comprehensive genomic analyses, and this improved availability has allowed several genomic profiling studies to be done for DLBCL. These investigations have revealed significant different mutational profiles for the GCB and ABC DLBCL subtypes.

GCB DLBCLs tend to have mutations to epigenetic modifiers such as enhancer of zeste homolog 2 (*EZH2*, a histone-lysine N-methyltransferase), CREB binding protein (*CREBBP/EB300*), and histone-lysine N-methyltransferase 2D (*KMT2D/MLL2*), as well as alterations to B-cell lymphoma 2 (*BCL2*). While mutations to *CREBBP* and *KMT2D* are also seen in patients with ABC DLBCL, those anomalies are more frequently observed in the GCB subtype. Hallmarks of the ABC DLBCL subtype include mutations resulting in altered chronic B-cell receptor and Toll-like receptor signaling, which can result in constitutive activation of different cellular signaling pathways. The nuclear factor kappa-B (NF-kB) pathway, in particular, is often activated in this manner.

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Genetic Biomarkers

Rearrangements of *MYC*, which are typically present in 5-15 percent of DLBCLs, have been shown in a number of studies to be a valid predictor of shorter survival.

"The *MYC* gene regulates a very wide array of cellular processes including growth and proliferation," explained Chan. "In the rearranged cases, *MYC* is usually involved in a translocation that fuses it to immunoglobulin heavy chain (IGH), which results in overexpression of MYC protein, which affects all of the pathways that MYC regulates, and this ultimately leads to rapid cell growth and proliferation."

Regarding other genetic biomarkers, Chan stated, "MYC overexpression, when associated with BCL2 expression, even without rearrangements, has been associated with higher risk DLBCL. These are so-called 'double expresser' DLBCLs. Other markers that have been associated with poor outcomes are CD5, high expression of p53, and EBER, which indicates an association with Epstein Barr virus infection."

In DLBCL, single mutations typically do not have any prognostic value; however, one potential exception to this is *TP53*. In an earlier study among 506 DLBCL patients receiving the R-CHOP regimen, it was shown that patients with DLBCL bearing *TP53* mutations had worse progression-free and overall survival compared with those without (*Blood* 2012;120(19):3986-3996). Importantly, *TP53* mutations were shown to have predictive value for R-CHOP–treated patients having either GCB or ABC subtype DLBCL.

The authors identified crucial structures for maintaining p53 function: the loop-sheet-helix and L3 motifs in the DNA-binding domain. Interestingly, loss of heterozygosity and TP53 deletion did not confer worse survival. In cases where mutational analyses were unavailable, patient stratification could be accomplished using IHC analyses which showed greater than 50 percent cells expressing p53 protein to provide populations with significantly different outcomes.

In a recent study of DLBCL patients, somatic copy number alterations and structural variants (SV) were integrated with recurrent mutations to identify five genetically distinct DLBCL subsets (*Nat Med* 2018;24(5):679-690). These subtypes included the following:

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• C1-a favorable risk ABC-DLBCL with extrafollicular genetic features, of possible marginal zone origin, having *BCL6* SVs combined with mutations in *NOTCH2* signaling pathway components;

• C2-a cell-of-origin-independent group of GBC-DLBCL and ABC-DLBCL tumors with frequent biallelic inactivation of *TP53* by mutations and 17p copy loss, as well as copy loss of 9p21.13/CDKN2A and 13q14.2/RB1;

• C3-poor risk, predominantly GCB-DLBCLs with *PTEN* alterations, SVs of *BCL2* and mutations to the epigenetic modifiers, *KMT2D*, *CREBBP*, and *EZH2*;

• C4-a newly defined group of predominantly GCB-DLBCLs with distinct alterations in BCR/PI3K, JAK/STAT and BRAF pathway components and multiple histones, making them potentially susceptible to BRAF/MEK1 and JAK/STAT blockade; and

• C5-a group of primarily ABC-DLBCLs increased BCL2 expression arising from *18q* gain with frequent mutations to MYD888 and CD79B that may have either CNS or testicular involvement.

DLBCL Discussion

"As far as targetable biomarkers go," Chan noted, "a few examples include newer generation anti-CD20 targeted drugs, brentuximab vedotin for targeting CD30, and venetoclax which targets BCL2, and there are newer antibody-based therapies targeting CD22 and CD79B. Immunotherapy has been a hot topic lately, and in this arena there are various checkpoint inhibitors and newer cellular therapies like CAR T cells.

"There have been a number of clinical trials with venetoclax, more than I can list off the top of my head, but major ones include usage of a first-line addition to R-CHOP or G-CHOP, as well as monotherapy for relapsed or refractory DLBCL, and in combination with other regimens."

When asked if there were any biomarkers associated with patients responding to R-CHOP therapy, Chan replied, "Right now it's not totally clear which patients will be curable by R-CHOP. The International Prognostic Index (IPI), which is a score based on a number of clinical parameters like age and stage, has historically been one of the best, if not the best predictor of outcome."

Regarding the current status of predictive biomarkers for DLBCL, Chan stated, "There aren't really definite biomarkers to predict outcome that everybody agrees on though, and most biomarkers that have been studied have not been able to predict outcomes independent of the IPI when put through rigorous statistical analysis."

Checkpoint Inhibitors

The use of monoclonal antibodies targeting the checkpoint molecules CTLA-4, PD-1, and PD-L1 has had a tremendous impact on the treatment of a number of solid tumor-bearing malignancies. However, the utilization of these powerful therapies in patients with hematologic malignancies has not progressed to the same degree.

In a recent review article, Djordje Atanackovic, MD, and Tim Luetkens, MD, from the University of Utah Huntsman Cancer Institute, discussed the application of several different checkpoint inhibitors in the treatment of patients with several different hematologic malignancies (*Semin Cancer Biol* 2018;52:198-206).

Diffuse Large B-Cell Lymphoma

Pidilizumab, an anti–PD-1 monoclonal antibody was evaluated in patients with DLBCL post-autologous stem cell transplantation (ASCT) in an international phase II study (NCT00532259). Of the 66 patients treated, 35 had residual disease post-ASCT; in these patients, the objective response rate (ORR) was 51 percent and the progression-free survival was 0.72 at 16 months after the first treatment. Interestingly, no predictive biomarkers were determined, although, increases in circulating lymphocyte subsets, including PD-L1+ activated helper T cells (e.g., CD4+PDL1+CD25+), were associated with treatment. These observations suggested an on-target in vivo effect for pidilizumab (*J Clin Oncol* 2013;31:4199-4206).

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Learning Objectives for This Month's CME Activity:

After participating in this CME/CNE activity, readers should be better able to: 1. Explain the latest findings about relevant genetic biomarkers for diffuse large B-cell lymphoma (DLBCL). 2. Analyze the application of several different checkpoint inhibitors in the treatment of patients with various hematologic malignancies.

Disclosure: The author(s), faculty, staff, and planners, including spouses/partners (if any), in any position to control the content of this activity have disclosed that they have no financial relationships with, or financial interests in, any commercial companies relevant to this educational activity.

Classical Hodgkin Lymphoma

In May 2016, the FDA granted accelerated approval to nivolumab, an anti-PD-1 monoclonal antibody, as a treatment for patients with classical Hodgkin lymphoma (cHL). This decision was stated to be based on results obtained in two clinical trials: a cohort containing patients with cHL in the phase I CheckMate 039 (NCT01592370) and the phase II CheckMate 205 (NCT02181738).

In one report on the CheckMate 039 study, an ORR of 87 percent was noted in a group of 23 heavily pre-treated patients with cHL (*N Engl J Med* 2015;372:311-319). For CheckMate 205, an ORR of 69 percent was noted in an extended follow-up of these patients (*J Clin Oncol* 2018;36(14):1428-1439). Participation in that trial was limited to patients with recurrent cHL who did not respond to ASCT, and who either did not respond to or relapse after responding to brentuximab vedotin.

A separate analysis of this study evaluated the relationships between objective response with genetic alterations of 9p24.1 and PD-L1 expression scores (*Lancet Oncol* 2016;17(9):1283-1294). Interestingly, every patient that attained a complete response had third or fourth quartile PD-L1 expression scores, while scores for patients experiencing progressive disease were in the first quartile.

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In March 2017, the FDA granted accelerated approval for pembrolizumab, another anti-PD-1 monoclonal antibody, for the treatment of adult or pediatric patients with relapsed or refractory classical Hodgkin lymphoma who had three or more prior lines of therapy. This approval was largely based upon the results obtained in the phase II KEYNOTE-087 clinical study (NCT02453594). That study included patients with relapsed/refractory cHL in three different cohorts: Cohort 1—those who experienced progression after ASCT and subsequent brentuximab vedotin; Cohort 2—those unable to achieve complete or partial response to salvage chemotherapy and did not receive ASCT, but have relapsed after treatment with or failed to respond to brentuximab vedotin; Cohort 3—those who failed to achieve a response to or progressed after ASCT and have not received brentuximab vedotin post ASCT. A 2-year follow-up of this study showed an ORR of 71.9 percent in the total study population.

Participants in the phase Ib KEYNOTE-013 study (NCT01953692), who had a number of different hematologic malignancies, were treated with pembrolizumab. Prior to treatment, biomarker analyses showed higher expression of PD-L1 and PD-L2 positivity. Analyses after pembrolizumab therapy showed that several biological changes occurred, including activation of interferon-γ, T-cell receptor, and expanded immune-related signaling pathways, as well as T-cell and natural killer cell expansion (*J Clin Oncol* 2016;34(31):3733-3739).

Chronic Lymphocytic Leukemia

One phase II study (NCT02332980) included 25 patients with relapsed or transformed chronic lymphocytic leukemia (CLL) who were treated with pembrolizumab; four of nine patients with Richter's transformation exhibited a clinical response and objective clinical responses were observed in four out of nine patients (44%). It is of interest to note that no clinical responses were observed in those participants with relapsed CLL. In contrast, patients who had confirmed responses displayed increased PD-L1 expression and enhanced PD-1 expression in the tumor microenvironment (*Blood* 2017;129:3419-3427).

Checkpoint Inhibitor Discussion

When asked to comment on how clinical studies of checkpoint inhibitors had gone in patients with hematologic malignancies, Atanackovic noted, "The most interesting finding has actually been that it is almost impossible to generalize. Certain subtypes of B-cell lymphomas, such as CLL and DLBCL, have shown relatively good response rates. Others, such as multiple myeloma (MM), which essentially represents a terminally differentiated B-cell lymphoma, have resulted in disappointing clinical responses. Unfortunately, the biological basis for these differences has remained unclear.

"To my mind, the most exciting finding with anti-PD-1/PD-L1 checkpoint inhibitors in hematologic malignancies was achieved in the treatment of patients with Hodgkin lymphoma," Atanackovic stated. "These patients have shown substantial clinical responses, a result that came somewhat unexpected.

"This finding is also very interesting from a biological perspective, because we have known for a while that in HL the tumor is surrounded by an inflammatory infiltrate that potentially has anti-tumor activity. Under normal circumstances, HL protects itself from these immune cells by suppressing them through immune checkpoints such as PD-1/PD-L1. That is something that it cannot do if you treat the patient with checkpoint inhibitors such as an anti-PD-1 antibody," he added.

When asked if there were differences between the CTLA4 and PD-1/PD-L1 antibodies' activities, Atanackovic replied, "Yes, absolutely. As with solid tumors, anti-PD-1/PD-L1 agents seem to be more clinically active than agents targeting CTLA4. Anti-PD-1/PD-L1 agents are probably also less toxic.

"Unfortunately, we do not have any reliable predictive biomarkers for most hematologic malignancies; DLBCL and Hodgkin lymphoma may represent exceptions. There is now sufficient data supporting PD-L1 expression levels in the tumor tissue as an independent prognostic factor in B-cell lymphomas such as DLBCL. Furthermore, expression levels of PD-L1 also seem to predict responses to anti-PD-1/PD-L1 approaches in patients with Hodgkin lymphoma." Future studies will have to further delineate the prognostic/predictive role of PD-L1 expression as a biomarker in hematologic malignancies.

When citing successes, Atanackovic stated, "Personally, I would say the greatest successes have been observed in those patients with Hodgkin lymphoma. That is because anti-PD-1 agents are clearly very active in this malignancy; as a tumor immunologist I am also enormously fascinated by the immune mechanisms behind the clinical responses in this hematologic malignancy.

"The most significant therapeutic opportunities are clearly in the area of combination therapies," he noted. "Combinations could include other checkpoint inhibitors, autologous or even allogeneic transplants, tumor-targeting monoclonal antibodies, and even cellular immunotherapies such as CAR T cells." OT

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Checkpoint Inhibition in Multiple Myeloma

In a recent conversation with *Oncology Times*, Djordje Atanackovic, MD, at the University of Utah Huntsman Cancer Institute, discussed some of the recent results from clinical studies evaluating checkpoint inhibitors in patients with multiple myeloma.

What were some of the problems that were encountered in earlier trials of checkpoint inhibitors in multiple myeloma?

Clearly, the most important problem with checkpoint inhibitors in multiple myeloma in very early clinical trials was a lack of efficacy. There were basically no clinical responses in clinical trials that used anti-PD-1 checkpoint inhibitors as single agents. Only when checkpoint inhibitors were combined with other immunomodulatory agents, such as lenalidomide, is when significant apparently clinical responses were observed. Obviously, these non-randomized and single-arm clinical trials were not able to delineate the role of each component in the overall outcome and, even more importantly, trials like this are not capable of identifying safety issues associated with the combination treatment versus each agent given separately.

What specifically led to the end of pembrolizumab being evaluated in multiple myeloma?

As explained in my article, despite the fact that the phase I/II clinical trial data looked very promising, two large randomized phase III clinical trials eventually put an end to the development of pembrolizumab in multiple myeloma. In July of 2017, the FDA placed a clinical hold on the pembrolizumab combination trials KEYNOTE-183 and KEYNOTE-185 in multiple myeloma. This decision followed a review of data by the data monitoring committee in which a lack of efficacy, as well as more deaths and more severe adverse events were observed in the pembrolizumab arms of KEYNOTE-183 and KEYNOTE-185. The FDA determined that the data available indicate that the risks of pembrolizumab plus pomalidomide or lenalidomide outweigh any potential benefit for patients with multiple myeloma. So far, no biomarkers with a predictive value for the increased toxicity have been identified.

Are there any other attempts to evaluate checkpoint inhibitors in the treatment of multiple myeloma and, if not, do you think that this will not be explored going forward?

Some of the trials that were temporarily on hold have reopened. I think that at the moment people are still somewhat hesitant to further investigate these agents in multiple myeloma. That is primarily because it is still unclear what the biological mechanisms were that led to the increased toxicity in the phase III trials. We definitely need to look into the mechanisms that resulted in these safety concerns. Only then will we be able to improve the safety, and maybe even efficacy, of checkpoint inhibitors in multiple myeloma. OT