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DLBCL, HGBL

ASCO 2017 | Diagnosis and treatment options for Double Hit Lymphoma – Educational session

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One of the opening sessions during the 2017 American Society of Clinical Oncology (ASCO) Annual Meeting was an Educational Session, which discussed the testing, treatment options, and patient perspective of Double Hit Lymphoma (DHL).

The session was chaired by Jason R. Westin, MD, MS, from The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, who described DHL as "one of the most challenging subjects in the world of Lymphoma".

Dr Westin began with an overview of NHL, explaining that it is a complicated disease with numerous subsets each with unique biology, which fall into two broad categories: indolent or aggressive. Approximately, 80,000 new cases are diagnosed each year in the USA and the most common involve B-cells and T-cells, although some subtypes involve Natural Killed (NK) cells.

Following this, the talked zoomed in to focus specifically on Diffuse Large B-Cell Lymphoma (DLBCL) and its subtypes outlined in the 2016 WHO classification. The classification which represents DHL is the "High-grade B-Cell Lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements" category. Jason Westin made the point that it is quite a challenge to explain what this is to a patient, and so the phrase "Double-Hit Lymphoma" is now used as it is much easier for patients to say and remember. When explaining DHL to a patient, Dr Westin gives the metaphor of a car, where the *MYC* translocation is where the gas pedal is stuck, and the *BCL2* translocation represents the brakes being cut.

Jason Westin finished his portion of the session by outlining a case study of a 39-year-old male who was healthy and active, and noticed some discomfort when wearing his bicycle helmet. Imaging was performed which discovered numerous nodal and non-nodal disease sites, and after biopsy it was determined that the patient had high-grade germinal center type DLBCL harboring *MYC* and *BCL2* translocations. At the MD Anderson Cancer Center, these patients are commonly treated with dose adjusted R-EPOCH, of which the patient was administered 6 cycles and responded well. Currently, there is discord on how to manage patients who achieve a Complete Response (CR) after 6 cycles of R-EPOCH. At the time, the MD Anderson Cancer Center protocol for young, fit patients was ASCT with BEAM and following this the patient was administered a regimen of lenalidomide and ipilimumab. Unfortunately, 6 months later the patient experience relapse with a very rapid, proliferative disease.

Lisa Rimsza, MD, from the Mayo Clinic Arizona, followed the opening portion of this session with a talk on DHL from "The Pathologist Perspective", mainly addressing the question of how DHL is diagnosed.

Dr Rimsza began by outlining the 2008 and 2016 classifications of DHL. In 2008, the category was titled "High-Grade B-Cell Lymphoma, Unclassifiable with Features Intermediate between DLBCL and Burkitt Lymphoma (BL)", or for short, B-Cell Lymphoma Unclassifiable (BCLU). These cases demonstrated an intermediate morphology between DLBCL and BL, cases that displayed BL morphology but harbored unusual phenotype (e.g. BCL2 IHC positive), or cases that had Double or Triple

Hits (represent approximately 1/3 of cases). This was a very difficult group due to the different classification methods: morphology, phenotype, or genetics. In the 2016 updated classifications, the BCLU group was separated into two different groups:

- High-grade B-Cell Lymphoma, Not Otherwise Specified (NOS): high-grade cytologies without DHL or Triple Hit Lymphoma (THL); must have non-DLBCL cytology
- High-grade B-Cell Lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements: all the DHLs and THLs that do not meet the specific criteria for other diagnoses (such as FL or LBL), irrespective of cytology

The first thing that should be done to determine a diagnosis of DHL is to look at the cytology of the disease. Currently, the diagnostic approach for high-grade BCLs relies heavily on morphology, and this presents numerous challenges for those in regular diagnostics: sometimes the sample is small, sometimes there are fixation issues, and sometimes the biopsy can be necrotic; all changing the way the disease looks. Dr Rimsza also covered the cytologic spectrum of DHL/THL: centroblastic, immunoblastic, anaplastic, spindle cell, and signet ring types of DLBCL can all be seen with DHL/THL genetics, as well as blastoid and DLBCL/BL high-grade types.

Following cytologic examination, assessment for translocations should be carried out. The *MYC* translocation is 8q24 and is absolutely required for DHL/THL diagnosis. Translocation partners are typically the immunoglobulin genes (approximately 75% of cases), and heavy chain is more common than κ or λ chains. Translocations with non-immunoglobulin partners occurs in around a quarter of cases. The question has been raised whether the type of translocation partner confers any prognostic relevance, immunoglobulin translocation patients may have worse outcomes. Overall, the frequency of the *MYC* 8q24 translocation is 15% of DLBCL cases and is more common in the germinal center subtype (19%) than the activated B-cell subtype (11%).

The *BCL2* translocation is 18q21 and partners with immunoglobulin heavy chain, or sometime light chain genes, in the majority of cases. Overall, this translocation occurs in 30% of DLBCLs, one again more common in the germinal center type (46%) than the activated B-cell type (5%).

Translocations of *BCL6* involve 3q27 and have numerous partner genes. This kind of translocation is observed in 23% of DLBCL patients, but compared to *MYC* and *BCL2* translocations, occurs more commonly in non-germinal center (30.1%) vs. germinal center subtypes (17.5%).

Dr Rimsza then gave an overview of DHL and THL by translocations present, percentage DHLs/THLs, and the cell of origin. Approximately, 5–10% of all *de novo* DLBCLs are DHL/THL.

Type	**Percentage of DHL/THL**	**Cell of Origin**
MYC and *BCL2*	80%	GCB
MYC and *BCL6*	10%	ABC or GCB

MYC and *BCL2* and *BCL6*	10%	GCB, possibly MUM1+

The focus of the talk then turned to what does not define DHL.

Abnormality	**Definition**
Extra copies/gains	Range from 3–10 copies of a certain gene; targeted gains, which exceed the overall ploidy; less likely to alter levels of mRNA than translocations
Amplifications	Targeted gains, which exceed the overall ploidy; Amplification of *MYC* or *BCL2* is observed in 20% (more in GCB) and 25% (more in ABC) of cases, respectively
Polysomy	>2 copies of chromosome including oncogene; likely less alteration of mRNA levels than gains/amplifications; by FISH, cannot distinguish from extra copies/gains unless chromosome enumeration probe used

Dr Rimsza then discussed Dual Protein Expression (DPE) of *MYC* and *BCL2* in DLBCL, and from her work it was noticed that *MYC* and *BCL2* dual expression does not appear to take place in CD20+ cells. From a pathology point of view, a small number of DPEs are also DHLs. Moreover, from a genetic perspective, most DHLs are DPEs, but not all (*MYC* is most often the discrepant). Currently, there are some issues with detecting *BCL2* expression as it may be affected by mutations or phosphorylation, and the cut-offs and intensity of *BCL2* expression detection is not agreed upon; positive generally is attributed to >50% of cells for *BCL2* and >40% of cells for *MYC.* In DLBCL, DPE of *MYC* and *BCL2* is observed in 21% of cases (22% GCB, 46% ABC).

Next, Laurie Sehn, MDCM, MPH, from the BC Cancer Agency, tackled DHL from "The Medical Oncologist Perspective", asking what data supports the therapy used for DHL patients.

In studies assessing the prognostic impact of DLBCL patients with *MYC* translocations (approx. 10–15% of patients), it appears they have a worse outcome than patients who do not harbor the translocation. However, in other studies, patients with dual expression of *MYC/BCL2* (approx. 30% of cases) have a modest outcome, slightly poorer than the general patient. It is becoming clear that dual expressor patients may be a significant area of interest that requires more investigation in clinical trials to determine the best treatment approaches and how to standardize *MYC* and *BCL2* expression. In patients with DHL with translocation of *MYC/BCL2* (approx. 5% of cases, relatively rare) have been demonstrated to have the worst outcome (in a study by Laurie Sehn's institution, none of these patients were alive beyond 7 months), and represent an area of high un-met need in DLBCL.

DHL patients represent a quite heterogeneous group of patients, as mentioned before, 5% are DLBCL but 20% are transformed FL, and some cases have BCLU. The type of translocations present can also vary, such as *MYC/BCL2, MYC/BCL6,* or THL, as well as differences in the *MYC* translocation partner as discussed in the previous section of the session. So far, no randomized trials are being conducted to guide treatment approached for DHL, and so most of the data discussed in Laurie Sehn's talk was retrospective and so may include an element of selection bias and not reflective of all-comers.

In an expansion study of 54 patients diagnosed at the BC Cancer Agency between 1991 and 2007 (Johnson *et al.* Blood. 2009):

- BCLU = 36 patients; DLBCL = 17 patients; FL = 1 patient; transformed = 43%
- R-CHOP administered to 34 patients; intensive therapy in 6 patients; palliative care in 14 patients
- Patients stratified by *de novo* or transformed disease has abysmal outcomes
- Patients with DLBCL (n=15) had better outcomes than those with BCLU (n=25): 3.0 years vs. 0.3 years ($P < 0.00001$)
- Patients with non-IG/*MYC* (n=17) had better outcomes than those with IG/*MYC* (n=23): 2.9 years vs. 0.3 years ($P = 0.0008$)

Based on early reports such as this one, many clinicians began to treat patients with regimens more commonly used for high-grade lymphoma such as BL, due to their aggressive biology and highly proliferative tumors. For example, the BC Cancer Agency adopted a BL-like approach as standard treatment for DHL patients: R-CODOX-M/IVAC with or without ASCT. When looking back at this experience, between 2003 and 2013, 25 patients had been treated (60% DLBCL, 40% BCLU) achieving a 2-year PFS was 47%. However, due to the highly refractory nature of patients' diseases, of the 25 patients, only 16 went on to undergo ASCT, and 2-year PFS in these patients was 60%.

The largest experience available is a retrospective study compiling data from 23 different centers in North America (n=311) of induction therapy for DHL by Petrich *et al.* published in Blood in 2014. Many patients displayed aggressive clinical characteristics such as stage III/IV disease (80%), elevated LDH (76%), extranodal disease (60%), bone marrow positivity (41%), and CNS positivity (7%). Most patients included had a combination of *MYC* and *BCL2* (87%), only 5% of patients had *MYC* translocation with *BCL6*. In the whole patient population, 2-year PFS was only 40% and 2-year OS was 49%. Moreover, patients treated with more aggressive regimens such as R-Hyper CVAD, DA-EPOCH-R, and R-CODOX-M/IVAC appeared to have better outcomes than patients treated with standard dose R-CHOP. Other conclusions of this study was that patients who achieved CR after induction did not benefit from SCT, and outcome was not affected by histology, type of dual translocation, or history or prior indolent Lymphoma. Poor risk features identified included advanced stage, LDH >3x upper normal limit, leukocytosis, and CNS involvement.

Another retrospective analysis of patients (n=129) diagnosed with DHL at the MD Anderson Cancer Center between 2003 and 2013 was published by Oki *et al.* in the British Journal of Haematology in 2014. In this analysis, patients with translocation and extra copies of *MYC* and *BCL2* and/or *BCL6* were included. By histology, 65% of patients had DLBCL, 27% had BCLU, 11% had transformed disease, and 8% were classified as "other". Overall, 3-year EFS was quite poor at 29% and 3-year OS was 38%. The cumulative incidence of CNS involvement was also calculated and found to be 13% at 3 years. When stratified by treatment, patients treated with more dose intensive regimens had better outcomes than those receiving standard dose R-CHOP.

Laurie Sehn then moved on to discuss further outcomes in patients who do achieve a CR after induction. A retrospective analysis of 159 patients in CR after R-CHOP or intensive therapy was published this year (Landsburg *et al.* JCO) and was reported on by the Lymphoma Hub in May. By histology, 71% of patients had DLBCL, 29% had BCLU, and 13% had transformed disease. ASCT was carried out in 39% of patients, with the remaining 61% not receiving ASCT. In patients who achieved CR after induction, 3-year RFS was 80% and 3-year OS was 87%, and it was concluded that improved outcomes are achieved with more intensive induction. However, there appeared to be no additional benefit achieved by undergoing ASCT; although Laurie Sehn stressed that this finding is based on retrospective data and is not a randomized comparison.

Next, this talk focused on DHL patients with R/R disease after ASCT, and discussed a study (Herrera *et al.* JCO. 2017) of 117 patients with chemotherapy sensitive R/R DLBCL or BCLU, of which 10% were double hit. In patients without DHL and those with DHL, 4-year PFS was 57% and 28%, respectively (*P* = 0.013); DHL independently associated with worse PFS and OS. This poor outcome indicates that there is a definite need for alternative therapeutic options in this group of patients.

Following this, outcomes according to the translocation partner of *MYC* was discussed. In a study of 774 *de novo* DLBCL patients treated with R-chemo by Copie-Bergman *et al.*, 32 patients had DHL. Of these, 19 had *MYC*/non-IG translocations and 12 had *MYC*/IG translocations. A poor outcome was only found in patients who harbored the *MYC*/IG rearrangement. Laurie Sehn pointed out that the Dako probes used in this study for *MYC* by FISH could have missed some of the rearrangements with non-IG partners and so requires further validation.

One last question is concerned with whether translocation of *MYC* with *BCL6* has the same impact as that with *BCL2*? Laurie Sehn stated that this is a challenging question to address due to the few numbers of patients who harbor the *MYC/BCL6* translocation (<20% of cases). Moreover, as previously discussed, DHL makes up approximately 5–8% of DLBCL; 90% of DHLs are GCB subtype with most of them being *MYC/BCL2* with or without *BCL6.* Only 10% of DHLs are ABC subtype, and these are nearly always *MYC/*BCL2. Therefore, the question addresses two biologically different groups of patients. A study by Ye *et al.*, published in Oncotarget in 2015, aimed to answer this question and included 898 *de novo* DLBCL patients who had received R-CHOP, of which 34 had DHL:

N=898	**GCB**	**ABC**	**All**
MYC/BCL2	19	1	20
MYC/BCL6	9	5	14

Using gene expression analysis, it was concluded that *MYC/BCL6* DHL patients are molecularly distinct from patients harboring *MYC/BCL2.* When comparing outcomes, only patients with *MYC/BCL2* had a poor OS and those with *MYC/BCL6* actually demonstrated an OS similar to the general DLBCL population. Again, Laurie Sehn stated that this requires validation in other trials.

In terms of a treatment algorithm, complete testing was advocated which should include FISH for *MYC/BCL2/BCL6,* Cell of Origin, and immunohistochemistry for *MYC* and *BCL2.* Following this, if patients have a "true" DHL (*MYC/BCL2)* and are fit then they should receive a more intensive therapy than R-CHOP, with many clinicians defaulting to DA-EPOCH-R as preferred approach. Laurie Sehn encouraged the administration of intrathecal methotrexate due to the high risk of CNS involvement. However, if patients have *MYC/BCL2* DHL and are unfit, or other patients such as those with *MYC/BCL6* or dual expressors, then they should receive R-CHOP.

Laurie Sehn concluded her portion of the session by stating that we require a better understanding of patient heterogeneity including the impact of different *MYC* translocation partners and the impact of other molecular aberrations and protein expression levels. Lastly, clinical trials of novel agents in this group of patients is a real top priority.

DHL from the "Translational Scientist's Perspective" was then discussed by David Scott, MBCHB, PhD, also from the BC Cancer Agency, and focused on the targets available to improve outcomes.

Based on 1,200 biopsies of DLBCL morphology from registry data and trials in the USA and Germany, and applying FISH and GEP for COO, approximately 12% of patients had *MYC* rearrangements, which were enriched in GCB subtype (around 40%). In addition, *MYC/BCL2* DHLs made up around 7% of DLBCLs and all cases were GCB; no cases of nearly 400 ABC type patients were found. Those with *MYC/BCL6* DHL made up only 1–2% of DLBCLs. Therefore, a total incidence of DHLs was found to be around 8% of DLBCLs.

It was asked how do we go beyond the current intensive regimens used to apply truly targeted treatment approached for DHL? Our current knowledge of pathways in DHL is very limited, and while we wait for the specific identification of aberrant pathways and vulnerabilities of DHL, it is reasonable to target pathways shared by GCB type DLBCL and BL, such as the PI3K-AKT-mTOR pathway.

David Scott then discussed the possibility of targeting *MYC* itself and began by giving an overview of how *MYC* works. The MYC protein dimerizes with MAX to create a transcription factor, which then un-pauses the RNA Pol II complex to transcribe certain genes et e-boxes, resulting in increased proliferation, apoptosis, and decreased differentiation. It has proved a challenge to find ways to target *MYC*. One approach is to use BET inhibitors which target BRD4, which regulates RNA Pol II, thereby disrupting the *MYC* transcription program. Additionally, BET inhibitors have been reported to reduce the expression of *MYC*. Other approaches include targeting *MYC* expression at the RNA and protein levels (e.g. such as HDAC inhibitors), disrupt the dimerization of MYC and MAX, agents which target other elements of the transcription complex, and inhibitors of downstream *MYC* effectors (e.g. Aurora Kinase inhibitors).

The other promising target for DHL treatment is *BCL2*, as we already have agents that have shown efficacy in other B-Cell Lymphomas, such as venetoclax. Attempts are now being made to incorporate *BCL2* inhibitors into front line treatment of DHL by combining them with intensive regimens; indeed, there is a phase I trial assessing venetoclax plus DA-EPOCH-R (NCT03036904).

Another approach is targeting B-cell surface markers with strategies such as Chimeric Antigen Receptor (CAR)-T cells and Bispecific T-Cell Engagers (BiTES), as after all DHL is a disease of B-cells. The focus of research now is to try and combine some of these agents to treat patients with R/R DHL such as PI3K and HDAC inhibitors. All the agents discussed by David Scott are currently on-trial and off-label.

The challenges and opportunities of DHL treatment were discussed. Firstly, treating R/R patients is an immense challenge and so there is an emphasis to shift more importance on first line treatment, which requires safe combinations with immuno-chemotherapy. Currently, it is unclear what the backbone of these regimens should be; whether R-CHOP should be used or DA-EPOCH R? Due to the rarity of DHL, every agent and every possible combination cannot be tested and so prioritization of novel agents is required. Moreover, how we gauge success of a novel regimen needs to be decided upon and defined.

David Scott followed this by discussing the impact of routine FISH testing, comparing data from Johnson *et al.* and Petriech *et al.* (discussed in Laurie Sehn's talk) with data from routine testing carried out in the BC Cancer Agency since 2014. Routine testing revealed a greater incidence of DLBCL morphology DHL (around 80%). Moreover, in numerous patients there were no triggering features present, they appeared as "garden-variety" DLBCL. David Scott then hypothesized that routine testing will result in improved outcomes for GCB as well as DHL patient groups; as currently, using historic comparators leads to an overestimate of trial success. Therefore, in answer to how we can gauge success of a novel regimen, David Scott stated that control groups and improved historic comparators are required, and the heterogeneity of DHL needs to be understood and appreciated, as mentioned earlier in the session. It is now estimated that more than 50% of translocations in DHL with *MYC* are with a non-IG partner. Non-IG partners include: *BCL6, LPP, PAX5, SOCS1*, and many others. However, probes currently used are designed for BL, and so miss approximately 40% of non-IG translocations.

Lastly, David Scott emphasized that the mutational landscape of DHL is largely unknown, and current studies have been very small looking at very target genes; we do not have genome or exome wide data, we do not know the cooperating mutations, and we do not know the clonal structure or evolution of DHL. We do know, however, that DHL has more genomic complexity than BL. Understanding the biology of DHL will help us understand the differences in heterogeneity, clinical presentation, and current as well as future treatment. Correlative studies will be crucial in the future to help understand why patients respond or not, and to define appropriate biomarkers.

Conclusion

At the end of the session Jason Westin continued with his case study of the 39-year-old male patient with DHL who relapsed after ASCT. As is has become apparent that clinical trials are the best weapon we have to fight DHL, the patient was enrolled onto one of the first clinical trials of CD19+ CAR-T cell therapy. The patient experienced mild cytokine release syndrome which manifested with headaches, fevers, and tachycardia; was treated with antibiotics. He was discharged on day 10, already displaying regression of disease. Now, 21 months after, there is still no evidence of any residual disease.

Jason Westin concluded the session by stating that DLBCL is fairly common, but DHL is only found if you look for it (test for *MYCL/BCL2/BCL6* translocations). The optimal therapy is currently unknown, including front line, consolidation, and relapse. Therefore, clinical trials are a top priority in order to understand and treat this disease.

References:

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