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Do we Really Need the Services of Next Generation Sequencing (NGS) in Diffuse Large B-Cell Lymphoma (DLBCL)?

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Abstract: *Diffuse large B-cell lymphoma (DLBCL) is the most frequent type of newly diagnosed patients with non-hodgkin lymphoma (NHL). Results were acceptable till the introduction of type I anti-CD20 (Rituximab) which resulted in a revolutionary improvement in both progression free and overall survival rates when combined with the standard chemotherapy (CHOP). Among the DLBCL patients, here is a subset of patients who does not respond to the standard immune-chemotherapy. Research concentrated on the cell of origin of DLBCL, the immuno-histochemistry along with the gene profile studies resulted in the differentiation between the germinal B-cell lymphoma (GCB) and the activated B-cell lymphoma (ABC); however, this finding did not result in better survival in the activated B-cell lymphoma which runs a bad course because DLBCL and ABC lymphoma are still treated with the same protocol. Efforts should be concentrated on discovering the key genes behind the pathogenesis and target the targetable genes rather accumulating data for nothing.*

1. Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) accounts for 30-40% of newly diagnosed cases of lymphoma (1). The fourth edition of Non-Hodgkin Lymphoma (NHL) classification of the World Health organization (WHO) stressed on the addition of new categories the double-hit (DHT) and triple- hit lymphoma (THL), which replaced the old category (the NHL unclassifiable with features between DLBCL and Burkitt's lymphoma). Other type is DLBCL without Bcl-2 and C-Myc or no Bcl-6 rearrangement (2). The double and triple hit lymphoma run worse course compared with DLBCL and do not respond to traditional immune-chemothrerapy. These new entities draw a gray zone lymphoma at the borderlines between them and the DLBCL (3). Double hit and triple hit lymphomas may have a germinal B-cell (GCB) or an activated B-cell (ABC) of origin. It is known that the prognosis is better in the GCB descent; however, the double/triple hit variants are demonstrating a bad response to treatment irrespective of the cell of origin (4). Reported data on the treatment of both DHL and THL is very limited and most studies are retrospective in origin except a prospective study published in the European hematologic Association (EHA) 2019 which demonstrated an improvement in both the progression free survival (PFS) and the overall survival (OS) to reach 82% and 77% for 5 years respectively by using a new dose intense immune-chemotherapy (5). In solid tumors, next generation sequencing (NGS) offered a revolutionary tool helping researchers to better understand the genetic alterations behind carcinogenesis and progression of cancers under treatment. Gene profile was used for the first time in lymphoma to differentiate between the GCB and ABS along with the immunohistochemistry which paved the road to several algorithms such as Hans algorithm which is widely adopted and it is comparable to the gene profile (6).

2. NGS Implication in DLBCL

NGS is an additive value in term of diagnosis, prognosis, sub-type classification and guiding treatment in DLBCL and NHL in general (7). DLBCL is a heterogenous disease characterized by a heterogenous genetic aberration. The former findings led Schmitz et al to postulate four genetic subtypes as follows: MCD group which is characterized by MYD88/CD79B genes comutations and N1 group (NOTCH 1 mutations) which are widely seen in ABC lymphoma, BN2 (NOTCH2 mutations and BCL-6 fusions) seen in both ABC and GCB lymphomas and the EZB (EZH2 mutations and BCL-2 translocations) which are documented in GCB lymphomas (8). The impact of this genetic subtype was profound on the response to the standard treatment since BN2 and EZH were

responsive to R-CHOP while MCD and N1 groups were not. The addition of somatic copy number alteration led to the formation of new 5 clusters as illustrated in table (1).

Table1. *illustrates the five clusters combining gene alterations as well as chromosomal aberrations (9)*

Cluster	Genetic aberration	Chromosomal aberration	risk
one	BCL-6 and NOCH2 comutations	NA	favorable
Two	Biallelic TP53 inactivation and CDKNA2 loss	NA	poor
Three	BCL-2 translocation, PTEN alterations and epigenetic mediators alterations: KMT2D, CREBBP and EZH2	NA	poor
Four	Alterations in BCR/PI3K, JAK/STAT and BRAF pathway	NA	favorable
five	BCL-2 and MALT-1 over-expression and CD79B/MYD88 mutations	18q gain	poor

Findings in table (1) shed light on the importance of gene profile study, which is a new sight to better classify DLBCL better than depending on the cell of origin (COO) studies; further, it guides researcher to tailor treatment depending on targetable genes or gene products giving the rational to use new generations of drugs. For instance, the BTK inhibitor Ibrutinib was added to chemotherapy to treat DLBCL patients with the MCD and cluster 5 groups (8, 9); further, patients with cluster 3 and 5 DLBCL are more sensitive to treatment with BCL-2 inhibitors and PI3Kα/δ blockade (10).

3. Correlation Between Identified Genes and Prognosis

LYSA trial is one of the most important examples on the role of NGS in new genetic profile discovery in DLBCL. Discovered genes were correlated with other prognostic factors including: age, stage, IPI score, bone marrow involvement and the presence of bulky disease (11). CD79B, KMTD2 and MYD88 mutations are correlated with old age as seen in ABC, which is more frequent in older patients (12). It was noticed that MYD88 mutation was correlated with high IPI score. B2M and the immunity pathway mutations were inversely correlated with the stage of disease while B2M and STAT mutations were find to be inversely correlated with IPI score. This was reflected in a decrease in both PFS and OS in ABC patients.

In the ABC DLBCL arm treated with R-CHOP, it was noted that patients with TNFAIP3 mutations are associated with shorter PFS and OS, while patients with GNA13 mutations are documented with short OS. However, this negative findings was not documented in younger patients with DLBCL harboring TNFAIP3 mutations where better when treated with the R-ACVBP protocol (13). Table (2) illustrates the mutational profile in B-cell NHL.

Table2. *mutational profile in B-cell NHL patients*

Lymphoma	Mutation profile	Biomarkers in use
DLBCL	BRAF, B2M, CARD11, CDKN2A, CD70, CD79a, CREBBP, EZH2, MF2B, NOTCH1/2, TP53	NA
GCB DLBCL	BCL2/BCL6, EZH2,GNA13, IRF8, MYC, SGK1, STAT3, TNFR14	NA
ABC DLBCL	CD79b, EP300, KMT2D, MYD88, PIM1, PRDM1	NA
PMBCL	B2M, NFKBIE, PTPN1,TNFAIP3, STAT6, XPO1	STAT6, XPO1
BL	ID3, TCF3, CCND3, TP53, CDKN2A, MYC, DDX3X, PTEN, PIK3R1, ARID1A, SMARCA4, GNA13, ROCK1	ID3, TCF3

ABC, activated B-cell; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; GC, germinal centre; LPL/WM, PMBL, primary mediastinal B-cell lymphoma

4. Clonality of B-Cell Lymphoma: Immunoglobuline is the Example

One of the most important methods in diagnosis of B-cell lymphoid malignancies is the clonal immunoglobulin (IG) gene rearrangement. In B-cell lymphoma, analyzing of IG heavy chain (IGH) and κ light chain (IGK) gene rearrangement is performed by means of EuroClonality/BIOMED-2 assay, which is considered the gold standard in this regard. IGH and IGK clonality studies are important in every case of suspected B-cell lymphoid malignancies because it is able to distinguish reactive from malignant tisues. These studies are helpful in assessment of relapsed clone in the same patients and detection of minimal residual disease (MRD as well (14).

5. Frequent Genes in DLBCL Discovered by NGS

Several genes play an important role in the pathogenesis of NHL while others are encountered mutated in some patients without a

definitive knowledge about their precise role in pathogenesis. Some genes play a role in diagnosis, prognosis and prediction of clinical response to immune-chemotherapy. The cancer genome project has studied most tumors including DLBCL illustrating the role of these genes and clinical trials recruiting patients with mutant genes. Table (3) illustrates the most frequent gene aberrations documented from patients with DLBCL.

Table3. *significant genes in NHL adopted from cancer genome: www.mycancergenome.org*

Gene	Incidence	Ongoing trials
ABL1	1.39%	5 trials
AKT1	0.82%	1 trial
ALK	2.48%	2 trials
APC`	3.18%	1 trial
ASXL1	3.17%	1 trial
ATM	5.65%	2 trials
ATR	1.53%	1 trial
BIRC3	1.85%	1 trial
BLM	1.57%	1 trial
BRAF	2.13%	2 trials
BRCA1	1.81%	4 trials
BRCA2	3.26%	4 trials
BRD4	0.73%	1 trial
CDK6	0.85%	1 trial
CDKN1A	0.85%	1 trial
CDKN1B	1.81%	1 trial
CDKN2A	10.16%	3 trials
DDR2	1.57%	1 trial
DICER1	1.09%	1 trial
DNMT3A	4.04%	1 trial
EGFR	2%	1 trial
ERBB2	1.18%	1 trial
ERBB3	1.33%	1 trial
EZH2	6.34%	1 trial
FBXW7	2.24%	1 trial
FGFR2	0.82%	1 trial
EGFR3	1.3%	2 trials
FLT1	0.97%	1 trial
FLT3	2.45%	7 trials
GATA3	O.85%	1 trial
HRAS	0.53%	1 trial
IDH2	1.94%	1 trial
KDM6A	1.54%	1 trial
KDR	1.88%	1 trial
KIT	1.81%	4 trials
KMT2A	0.66%	2 trials
KMT2D	24.97%	1 trial
KRAS	3.63%	1 trial
MAP3K1	1.45%	1 trial
MDM2	0.73%	1 trial
MECOM	2.83%	4 trials
MED12	3.71%	1 trial
MET	2%	3 trial
MLH1	1.18%	1 trial
MLH3	3.2%	1 trial
MSH2	1.45%	2 tials
MSH6	1.81%	2 trials
MTAP	5.02	1 trial
MTOR	3.02%	1 trial
MYC	4.72%	3 trials
NF1	1.81%	1 trial

NOTCH1	6.22%	2 trials
NOTCH2	4.11%	2 trials
NOTCH3	2.85%	1 trial
NRAS	3.74%	1trial
NSD1	3.49%	1 trial
NTRK1	2.3%	3 trials
NTRK2	1.33%	2 trials
NTRK3	0.6%	1 trial
PDGFRA	0.96%	1 trial
PIK3C2B	1.95%	1 trial
PIK3CA	1.18%	1 trial
PIK3R1	1.21%	1 trial
PMS1	0.73%	1 trial
PMS2	0.87%	1 trial
POLE	1.53%	1 trial
PTCH1	1.21%	2 trials
PTEN	2.35%	1 trial
PTPN11	1.49%	1 trial
RB1	3.42%	1 trial
RET	2.12%	1 trial
RUNX1	1.97%	1 trial
SETD2	3.75%	1 trial
SF3B1	1.31%	1 trial
SMARCA4	2.9%	2 trials
SMARCB1	0.83%	1 trial
SMO	0.71%	1 trial
SOX9	0.61%	1 trial
STAG2	1.21%	1 trial
TET2	9.73%	1 trial
TP53	13.34%	8 trials
TSC1	1.215	1 trial
TSC2	2.78%	1 trial
XPO1	2.9%	1 trial

6. Conclusion

With the introduction of immune-chemotherapy combination, the treatment of DLBCL became more effective and the survival rates were improved as well (15). In a subset of patients who do not respond, type II anti-CD20 (Obinutuzumab) demonstrated good results (15). Gene profile along with the immune-histochemistry has led to a better understanding at the level of the cell of origin and led to the differentiation between ABC and GCB lymphomas; however, the gene profile was comparable to immune- histochemistry by using several developed algorithms (6). Gene profile studies helped researchers and physicians to sub-classify DLBCL into 5 clusters and every sub-type has its own genetic aberrations which can predict response to treatment and prognosis as well. Gene studies led to the differentiation between double hit and triple hit lymphoma; however, the gene profile studies has not reflected yet into better improvement in treatment results which can be attributed to several factors such as the difficulty of finding a targetable gene or a gene product, the heterogeneity of genetic aberrations and the delay in clinical trials results which is a multi-steps time consuming work. Finally, NGS studies on DLBCL patients will pave the road for much serious precise medicine through discover of new gene aberrations and link them with new generation of drugs.

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