Next-Generation Sequencing in Diffuse Large B-Cell Lymphoma Highlights Molecular Divergence and Therapeutic Opportunities: a LYSA Study

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Abstract

Purpose: Next-generation sequencing (NGS) has detailed the genomic characterization of diffuse large B-cell lymphoma (DLBCL) by identifying recurrent somatic mutations. We set out to design a clinically feasible NGS panel focusing on genes whose mutations hold potential therapeutic impact. Furthermore, for the first time, we evaluated the prognostic value of these mutations in prospective clinical trials.

Experimental Design: A Lymphopanel was designed to identify mutations in 34 genes, selected according to literature and a whole exome sequencing study of relapsed/refractory DLBCL patients. The tumor DNA of 215 patients with CD20+ de novo DLBCL in the prospective, multicenter, and randomized LNHL-03B LYSa clinical trials was sequenced to deep, uniform coverage with the Lymphopanel. Cell-of-origin molecular classification was obtained through gene expression profiling with HGU133+2.0 Affymetrix GeneChip arrays.

Results: The Lymphopanel was informative for 96% of patients. A clear depiction of DLBCL subtype molecular heterogeneity was uncovered with the Lymphopanel, confirming that activated B-cell-like (ABC), germinal center B-cell like (GCB), and primary mediastinal B-cell lymphoma (PMBL) are frequently affected by mutations in NF-kB, epigenetic, and JAK–STAT pathways, respectively. Novel truncating immunity pathway, ITTPKB, MFIHAS1, and XPO1 mutations were identified as highly enriched in PMBL. Notably, TNFAIP3 and GNA13 mutations in ABC patients treated with R-CHOP were associated with significantly less favorable prognoses.

Conclusions: This study demonstrates the contribution of NGS with a consensus gene panel to personalized therapy in DLBCL, highlighting the molecular heterogeneity of subtypes and identifying somatic mutations with therapeutic and prognostic impact.

See related commentary by Lim and Elenitoba-Johnson, p. 2829

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common form of adult lymphoma worldwide, accounting for 30%–40% of newly diagnosed non-Hodgkin lymphoma (NHL; ref. 1). Gene expression profiling (GEP) has made strides in deciphering the molecular heterogeneity of DLBCL, enabling the entity's subdivision into three main molecular subtypes: germinal center B-cell like (GCB), activated B-cell like (ABC), and primary mediastinal B-cell lymphoma (PMBL; refs. 2, 3). Among other genetic aberrations, the GCB subtype is characterized by t(14,18)(q32;q21) translocations (4) and loss of PTEN (5), while the ABC subtype is characterized by t(3;14)(q27;q32) translocations (4) and deletion of the SOCS1 (6). Arising from B cells at distinct stages of differentiation and maturation, these subtypes are also diverse in clinical presentation, response to immunochemotherapy, and outcome, with the ABC subtype having the most unfavorable prognosis (2, 7). As targeted therapies become increasingly widespread, it is essential to thoroughly characterize each molecular

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Translational Relevance

This is the first next-generation sequencing (NGS) study of such a large, prospective cohort using a targeted gene panel, the Lymphopanel, focusing on genes identified as important for lymphomagenesis or whose potential has been pinpointed in whole exome sequencing studies. Particular attention has been paid to the inclusion of actionable targets in the Lymphopanel, highlighting potential candidate patients for novel personalized therapies. This study further details subtype-enriched gene and pathway mutations to optimize DLBCL treatment, and highlights several novel, frequent, and actionable mutations, notably in PMBL. In addition, Lymphopanel NGS has enabled the detection of novel clinical and prognostic correlations, potentially impacting treatment decisions. As benchtop sequencers become increasingly available in academic research and routine clinical settings, our study demonstrates the feasibility and impact of NGS with a consensus gene panel in DLBCL patient management, contributing essential information to today’s precision therapy era treatment decisions.

Materials and Methods

Patients

215 adult patients with de novo CD20⁺ DLBCL enrolled in the prospective, multicenter, and randomized LNH-03B LYSA trials with available frozen tumor samples, centralized histopathologic review, and adequate DNA/RNA quality were selected. The LNH03-B LYSA trials were initiated in 2003 and included 1,704 patients overall in six distinct clinical trials (Supplementary Methods). COO molecular classification was obtained with HGU133+2.0 AffymetrixGeneChip arrays (Affymetrix), grouping patients into ABC, CCB, PMBL, and "other," also referred to as unclassified (detailed in Supplementary Methods). Clinical features of the patients are indicated in Supplementary Table S1. The study was performed with approval of an Institutional review board and written informed consent was obtained from all participants at the time of enrollment.

NGS experiments and data analysis

The Lymphopanel was designed to identify mutations in 34 genes important for lymphomagenesis, based on literature data (Supplementary Table S2; ref. 24) and WES of relapsed/refractory DLBCL sequencing (25). The design covers 87,703 bases using 872 amplicons. Genes were grouped into 8 specific pathways: Immunity (CIITA, B2M, TNFRSF14, and CDS8), NOTCH (NOTCH1 and NOTCH2), Apoptosis/Cell cycle (MTHAS1, XPO1, MYC, CDKN2A/B, FOXO1, TP53, GNA13, and BCL2), NFKB (TNFAIP3, MYD88, PIM1, CARD11, IRF4, and PRDM1), Epigenetic Regulation (EZH2, KMT2D, EP300, MEF2B, and CREEBBP), MAP Kinase (BRAF), IAK-STAT (SOCS1 and STAT6), and BCR (CD79A/B, ITPKB, TCF3, and ID3; Supplementary Table S3).

Ion Torrent Personal Genome Machine (PGM) Sequencing and PGM data analysis was performed as described previously (26) and detailed in Supplementary Methods. Variant analyses were performed using an in-house generated bioinformatics pipeline, detailed in Supplementary Methods and Supplementary Fig. S1 and S2. This pipeline ensured excellent filtering of artefacts and polymorphisms, as proven by an independent study of seven nontumoral samples from DLBCL patients with a mean error rate of nonfiltered variants of only 0.7% (Supplementary Table S4).

Statistical analysis

All statistical analyses were performed using R software version 3.1.2 (27). Progression-free survival (PFS) was evaluated from the date of enrolment to the date of disease progression, relapse, retreatment, or death from any cause. Overall survival (OS) was evaluated from the date of enrolment to the date of death from any cause. Multivariate Cox and log-rank tests ("survival" R package version 2.37.7) were used to assess differences in OS and PFS rates calculated by Kaplan–Meier estimates. Ward hierarchical unsupervised clustering ("LPS" R package version 1.0.10) was performed using Jaccard distance. Statistical differences between all other parameters were determined using χ², Mann–Whitney, or Fisher exact tests when appropriate. P values...
or false discovery rates (FDR) < 0.05 were considered statistically significant.

Results and Discussion

Lymphopanel variant features

Lymphopanel NGS was performed on 215 DLBCL patients (81 ABC, 83 GCB, 33 "other," and 18 PMBL). The median overall sequencing depth was 225x (110x-331x). An initial 13,965 variants were filtered for quality, SNPs, and functional relevance, leading to 1,064 (7.6%) variants ultimately validated (Supplementary Table S5 and Supplementary Fig. S1). The Lymphopanel informativity was of 96%, with 206 patients (78 GCB, 80 ABC, 30 "other," and 18 PMBL) presenting at least one variant. All genes in the Lymphopanel were informative for at least one patient, with the number of variants per gene ranging from one to 124. Gene mutation frequencies in the total cohort and by subtype are presented in Fig. 1.

We subdivided the genes targeted by the Lymphopanel into eight specific pathways (Fig. 2; Supplementary Table S3). As expected, we confirmed that the ABC subtype is dominated by NFκB pathway mutations (45% of total variants), followed by epigenetic regulation pathway mutations (20.2%) of total variants; Fig. 2A), whereas the GCB subtype is mostly characterized by mutations in the epigenetic regulation pathway (32.3% of total variants) and the apoptosis/cell-cycle pathway (25.9% of total variants; Fig. 2B). Mutations in the JAK–STAT, immunity, and apoptosis/cell-cycle pathways were prevalent in PMBL (29.1%, 20.9%, and 20.9% of total variants, respectively; Fig. 2C). Interestingly, PMBL presented a significantly higher number of variants per sample than the other subtypes combined ($P = 2.76 \times 10^{-5}$, Supplementary Fig. S3). The "other" subtype showed a fairly hybrid spectrum of pathway mutations (Fig. 2D), perhaps suggesting the involvement of the three main subtypes, rather than the existence of a distinct entity.

We sought to assess the impact of AID involvement in the Lymphopanel genes (detailed in Supplementary Methods and Supplementary Fig. S4). Genes with an AID mutation frequency superior to the average of that of the Lymphopanel are presented in Supplementary Table S6. Previously identified SHM targets in DLBCL among these genes include MYC, PIM1, IRF4, BCL2, SOCS1 and CIITA (23). Potentially novel SHM targets include MFHAS1, PRDM1, GNA13, MYD88, ITPKB, and NOTCH2. There was no significant difference in AID involvement between DLBCL subtypes.

Lymphopanel NGS identifies mutations with potential treatment impact

The Lymphopanel included genes whose mutations could serve to stratify patients according to treatment options. This includes actionable mutations, currently targeted by precision therapies, highlighted in Fig. 1, as well as mutations whose presence might call into question the use of certain targeted therapy options. Recent results have shown that doubly mutated MYD88/CD79B patients are significantly more responsive to single therapy ibrutinib, a BTK inhibitor, highlighting the importance of targeted NGS for DLBCL patients in the clinical setting (28). Furthermore, the presence of CARD11 and TNFAIP3 mutations has been shown to lead to decreased activity of both ibrutinib and sotrastaurin, a protein kinase C inhibitor (29, 30). MYD88 mutations are frequent in ABC DLBCL, L265P being the most prevalent variant (up to 29% in previous studies; ref. 18). In our cohort, MYD88 mutations were significantly enriched in ABC patients (28.4%, FDR = 4.7×10^{-3}), with the L265P mutation present in 21% of ABC patients (Fig. 3 and Supplementary Fig. S5A). Of 17 ABC patients with MYD88 L265P mutations, we found 10 (58.8%)
with associated CD79B mutations, who might respond more favorably to ibrutinib treatment. CD79B mutations were identified in 10.7% of total patients, slightly less than previously reported, but were significantly enriched in ABC DLBCL (24.7%, FDR = 3.2 × 10⁻⁵), corroborating previous studies (13, 31). Of note, two (11.8%) MYD88 L265P–mutated ABC patients were CARD11-mutated and two were TNFAIP3-mutated, potentially diminishing the effect of ibrutinib and sotrasutin, while 6 (35.3%) were PRDM1-mutated (Supplementary Fig. S6).

ABC cells have been shown to be addicted to IRF4 activity (32), responsible for lenalidomide treatment response. Twenty-one IRF4 variants were discovered in 7.9% of patients (13.6% of ABC, 4.8% of GCB, 0% of “other,” and 11.1% of PMBL; Fig. 1). Although the impact on expression of these mutations was not analyzed, only one IRF4 variant was a stop-gain mutation, indicating that expression is most likely at least conserved. Most of the nonsynonymous SNVs were clustered around amino acid positions 18 (two S18 variants and two G20 variants) and 99 (four C99R variants, SIFT scores=0; Fig. 4).

Interestingly, IRF4 mutations were almost equally frequent in PMBL and ABC in our cohort. Although IRF4 addiction has not been demonstrated in PMBL, this predilection for IRF4 mutations, added to the well-known high expression of IRF4 in this subtype, may suggest a potential role for lenalidomide treatment in this subtype as well, calling for further investigation of these mutations’ effects.

Recently, small-molecule inhibitors of PIM kinases have been developed and studied in DLBCL. In our cohort, PIM1 mutations were significantly skewed toward ABC (33.3%, FDR = 3.3 × 10⁻⁵) in our cohort and represented 20.2% of ABC variants (Figs. 2A and 3 and Supplementary Fig. S5A). Of note, truncating PIM1 variants were predominant in ABC patients (13/14 identified). Interestingly, DLBCL sensitivity to PIM kinase inhibitors is not correlated with the level of PIM kinase expression (33), suggesting that even expression-modifying PIM1 mutations might not necessarily hamper the efficacy of PIM1 inhibitors, although this remains to be verified. In any case, the high frequency of PIM1 mutations in our cohort seems to justify the prescreening of PIM.
kinase inhibitor candidate patients by PIM1 sequencing, to identify those most likely to benefit from this targeted therapy.

The most highly mutated gene in GCB, BCL2 (34), can notably be targeted by BH3-mimetics. Among our GCB cohort, BCL2 was the third most frequently mutated gene (24.1%, FDR = 2.5 × 10^{-3}; Fig. 3 and Supplementary Fig. S5B), with mutations most frequently impacting the amino terminus and between the BH1 and BH3 domains. The BH3 domain was not impacted, indicating that BH3 mimetic activity would not be hampered in these patients (Supplementary Fig. S7).

Targeting epigenetic changes is also being increasingly explored in the realm of tailored therapy, notably among the GCB subtype, justifying the inclusion of genes such as EZH2, CREBBP, KMT2D, and EP300 within the Lymphopanel. In our cohort, 18.1% of GCB patients were EZH2 mutated, mostly at the Y641 hotspot, and EZH2 mutations were enriched in GCB (FDR = 1.8 × 10^{-3}), as observed in previous studies, with no EZH2-mutated ABC patients (ref. 13; Fig. 3 and Supplementary Fig. S5B). Interestingly, one PMBL patient presented an EZH2 Y641 mutation, adding to the debate of which DLBCL subtypes might benefit from EZH2 inhibitor treatment (26). CREBBP mutations were identified in 31.3% of GCB patients and 6.2% of ABC patients, corroborating previous studies (17), but were also found in 24.2% of “other” and 11.1% of PMBL (Fig. 1 and Supplementary Fig. S5). KMT2D mutations, evenly distributed throughout the coding sequence, were identified in 40.9% of our cohort, with KMT2D being the most frequently mutated gene in all subtypes except PMBL (Fig. 1). Of the 124 variants, 78 were truncating mutations, indicating bias toward loss of protein expression.

Mutations of master regulators TP53 and MYC showed no significant difference in subtype distribution in our cohort (Fig. 3).
TP53 variants were present in 14.9% of total patients (slightly less than previously reported; refs. 13, 35; Fig. 1) and several recurrent variants were identified, including six R248 variants, shown to be loss-of-function mutants in DLBCL (35). As for MYC, we discovered mutations in 6.5% of total patients (4.9% of ABC, 9.6% of GCB, 3% of "other," and 5.6% of PMBL). No mutations were
identified at the known hotspots in Burkitt lymphoma, including S62 and T58.

Finally, although frequent mutations are most enticing for therapeutic potential, rare mutations can also be potentially very effectively actionable. For instance, BRAF and NOTCH1 mutations have been identified as rare but functionally relevant in DLBCL (13). In our cohort, we identified one patient with an activating V600E BRAF variant. As for NOTCH1, 10 patients in our cohort presented mutations (Fig. 1). TCF3 was mutated in three patients, all of whom harbored a pathogenic N551K variant, which has shown altered DNA-binding specificity (36). The negative regulator of TCF3, ID3, was mutated in 4.7% of patients (Fig. 1), with most variants affecting the HLH domain (Supplementary Fig. S7), potentially impairing its ability to inactivate TCF3, as shown in Burkitt lymphoma (36). ID3 and TCF3 mutations have recently been shown to be of comparable frequency in Burkitt lymphoma and DLBCL, highlighting the overlap between these two entities (37).

The Lymphopanel highlights a unique PMBL mutational signature

STAT6 and SOCS1 mutations were both very significantly enriched in PMBL (FDR = 6.8 × 10^-14 and FDR = 2.5 × 10^-3, respectively), with STAT6 being the most frequently mutated gene in PMBL (72.2%, Fig. 3 and Supplementary Fig. S3C). All STAT6 mutations were nonsynonymous SNVs and recurrent variants were frequent among PMBL, including seven N417 variants (Supplementary Fig. S7). SOCS1 mutations were present in 55.5% of PMBL patients, 30% of them harboring SOCS1-inactivating mutations (Supplementary Fig. S7). TNFAIP3 mutations have also been frequently identified in PMBL, up to 36%; ref. 38. We found TNFAIP3 mutations to be significantly enriched in PMBL (61.1%, FDR=3.1×10^-3), with all but one TNFAIP3-mutated PMBL patient harboring truncating mutations (Fig. 3 and Supplementary Fig. S5C). The frequent TNFAIP3 and SOCS1 mutations in PMBL highlight the similarities between PMBL and cHL (38, 39).

Furthermore, no MYD88, CD79B, or CARD11 mutations were observed in PMBL patients (Fig. 1), suggesting a PMBL dependence on TNFAIP3 mutations for the constitutive activation of NF-κB. Interestingly, significant GNA13 mutation enrichment was shown in PMBL rather than GCB in our cohort (50%, FDR = 2.8 × 10^-4; Fig. 3 and Supplementary Fig. S5), and one third of GNA13 variants were potentially truncating mutations (Fig. 4).

We discovered 46 B2M variants in 18.1% of total patients, significantly enriched in PMBL (50%, FDR = 1.2 × 10^-5; Figs. 1 and 3 and Supplementary Fig. S3C). Six of 9 B2M-mutated PMBL patients harbored truncating mutations and the remaining three presented pathogenic nonsynonymous SNVs, two of which were a highly recurrent p.M1R variant predicted to affect the start codon (Fig. 4). Recently, frequent B2M mutations leading to lack of protein expression were identified in cHL (9). Our similar findings in PMBL further highlight the molecular similarities between these two entities. CD58 variants were also significantly enriched in PMBL (FDR = 1.2 × 10^-3; Fig. 3 and Supplementary Fig. S5), with 5 of 7 CD58-mutated PMBL patients exhibiting truncating mutations, potentially leading to a lack of CD58 expression. Importantly, PD-1 blockade has recently shown promise as an effective treatment of relapsed or refractory cHL (40). Escape from T-cell immunity via B2M and/or CD58 mutations could potentially affect the activity of PD-1 inhibitors in PMBL and cHL and warrants further investigation. Furthermore, we found 39 CIITA variants, enriched in PMBL as well (55.6% of PMBL vs. 14.1% of total patients, FDR = 3.1 × 10^-5; Figs. 1 and 3 and Supplementary Fig. S5C). To our knowledge, this is the first study to identify truncating immunity pathway mutations as major in PMBL, although CIITA/PDL1-2 translocations had been shown to impact PMBL survival (41).

ITPKB, MFHAS1, and XPO1 mutations were identified as significantly overrepresented in a relapsed/refractory DLBCL patient cohort (25), leading to their inclusion in the Lymphopanel. In our cohort, ITPKB, MFHAS1, and XPO1 mutations were all identified for the first time as being significantly enriched in PMBL patients (FDR = 1.4 × 10^-2, FDR = 3.9 × 10^-3, and FDR = 4.8 × 10^-10, respectively; Fig. 3). The roles of ITPKB and MFHAS1 in lymphomagenesis are not yet fully elucidated. ITPKB regulates B-cell survival and function, and ITPKB deficiency leads to B-cell antigen presentation unresponsiveness and decreased B-cell survival, associated with specific overexpression of proapoptotic Bim protein (42). We found 48 ITPKB variants, present in 14% of total patients (Fig. 1 and Supplementary Fig. S5). ITPKB mutations were uniformly distributed along the protein sequence, excepting the seemingly conserved catalytic IPK domain, affected by only two nonsynonymous SNVs (Fig. 4). MFHAS1 is a potential oncogene with highly conserved leucine-rich tandem repeats, isolated from a minimal common amplification region in malignant fibrous histiocytomas at 8p23.1. MFHAS1 is also a target gene for chromosomal translocation (8;14)(p23.1;q21) in B-cell lymphoma cell lines and has been shown to be tumorogenic in nude mice (43). In our cohort, MFHAS1 variants were identified in 7.9% of total patients, and were distributed homogeneously along the coding sequence, with no recurrent mutations identified.
Although the role of these potential oncogenes in lymphomagenesis is still unclear, their high mutation frequency in PMBL especially warrants further investigation.

As for \textit{XPO1}, it encodes CRM1, an exporter of several tumor suppressor proteins (TSP). Cytoplasmic export of TSPs renders them inactive, indicating that \textit{XPO1} acts as a proto-oncogene. \textit{XPO1} mutations were present in 4.7% of total patients (1.2% of ABC, 1.2% of GCB, 3% of “other,” and 38.9% of PMLB; Fig. 1 and Supplementary Fig. S5). The mutation identified is recurrent, with all variants affecting E571 (Fig. 4), as previously found in rare CLL cases (<5%; ref. 44). Importantly, selective inhibitors of nuclear export (SINE) have been shown to effectively target CRM1 and retain TSPs in the nucleus (45). Given the frequency of \textit{XPO1} E571 mutations in PMLB, further investigation concerning their impact on response to SINEs is warranted (46).

Identification of gene mutations correlated with clinical characteristics and prognosis

We tested each gene in the Lymphopanel for correlation with clinical characteristics including age, stage, IPI, bone marrow involvement, and the presence of bulky disease.

\textit{CD79B}, \textit{KMT2D}, and \textit{MYD88} mutations were significantly correlated with age (Supplementary Fig. S8). For \textit{CD79B} and \textit{MYD88} mutations, enriched in ABC, this might be linked to the significantly higher age of ABC patients \((P = 1.01 \times 10^{-2})\) in our cohort; ref. 47. As for \textit{KMT2D} mutations, homogenous among subtypes, this result potentially suggests an accumulation of passenger-type mutations, particularly visible in this long gene \((16.6 \text{ kb sequenced})\). \textit{MYD88} mutations were also significantly correlated with higher IPI \((\text{FDR} = 0.04)\).

On the other hand, PMLB patients were significantly younger \((P = 6.6 \times 10^{-5})\) and the large majority of genes whose mutations were significantly enriched in PMLB were also correlated with younger age (Supplementary Fig. S8). In addition, \textit{B2M} and immunity pathway mutations were significantly inversely correlated with Ann Arbor stage \((\text{FDR} = 3.2 \times 10^{-4} \text{ and FDR} = 0.045 \text{ respectively})\), and \textit{B2M} and \textit{STAT6} mutations were significantly inversely correlated with IPI \((\text{FDR} = 5.5 \times 10^{-4} \text{ and FDR} = 0.026, \text{respectively})\).

As expected, OS and PFS decreased with increasing IPI and ABC patients displayed significantly worse OS and PFS than GCB patients in the R-CHOP treatment group \((P = 1.9 \times 10^{-2} \text{ and } P = 6.6 \times 10^{-3}, \text{respectively})\). Of note, PMLB patients in our cohort did not present the favorable prognosis typically associated with this subtype (48), perhaps due to the lack of dedicated treatment.

Prognostic impact was assessed for all Lymphopanel genes and subtypes, among patients treated with R-chemotherapy, separated into an R-CHOP group and an R-ACVBP group. Analyses over all subtypes were performed using multivariate Cox tests, while analyses of each subtype individually were performed using log-rank tests. In both cases, independent corrections of \(P\) values were applied.
were performed for OS and PFS. Gene mutations harboring prognostic impacts with uncorrected \( P \) values < 0.05 are highlighted in Table 1 and Supplementary Fig. S10, and all survival analyses are presented in Supplementary Table S7. We set an FDR threshold of 0.05 to detect gene mutations with significant prognostic impacts.

Among ABC DLBCL treated with R-CHOP, TNFAIP3 mutations were significantly associated with lower OS (FDR = 7.86 \times 10^{-4}) and PFS (FDR = 3.04 \times 10^{-2}), while GNA13 mutations were significantly associated with lower PFS (FDR = 3.04 \times 10^{-2}; Fig. 5). Of note, the poor prognostic impact of these mutations was not observed in patients treated with R-ACVBP, which, for TNFAIP3 at least, might be linked to the improved survival observed in younger patients with DLBCL treated with R-ACVBP (49). These findings need to be confirmed in an independent cohort of patients, given the small sample size.

Unfortunately, the prognostic impact of TNFAIP3 mutations in PMBL patients could not be assessed due to the existence of only one WT TNFAIP3 PMBL patient treated with R-CHOP (Supplementary Table S7). Moreover, previously described prognostic impacts of TP53, FOXO1, or MYD88 mutations were not confirmed (35, 50, 51), although the impact of specific deleterious mutations was not analyzed on its own.

In conclusion, NGS is increasingly accessible in academic research settings, but its possibilities in routine clinical settings have yet to be thoroughly harnessed. This study has shown that, by using a restricted set of genes, not only can well-known mutations be tracked, but novel or rare mutations can also be identified and potentially targeted as well. Detailing subtype-enriched gene and pathway mutations is critical for optimal understanding and treatment of DLBCL: here, we have added to the field’s current knowledge of the genetic heterogeneity between DLBCL subtypes, and identified novel clinical and prognostic correlations, which might also have an impact on therapeutic decisions. NGS with a consensus targeted panel could contribute valuable information to multidisciplinary meetings and constitute a molecular breakthrough in the way treatment decisions are made, by providing personalized mutational profiles of actionable targets. Furthermore, although this study was performed on frozen tumor samples, which are of limited availability, the Lymphopanel was designed to be effective in FFPE samples as well, further cementing its capacity to play an essential role in clinical disease management. In addition, our team has recently demonstrated that Lymphopanel NGS can be successfully performed using plasma-extracted cell-free circulating DNA, highlighting its feasibility and potential role in the monitoring of DLBCL (52). Taken together, these results serve as proof-of-principle that NGS with a consensus gene panel can alter the manner in which DLBCL patients are subclassified and treated.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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