Original Article

Mutational Analysis of EZH2 in the Germinal Centre Type of Diffuse Large B Cell Lymphoma

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ABSTRACT

OBJECTIVE: To determine the Frequency of Germinal Center B (GCB) type Diffuse Large B Cell Lymphoma (DLBCL) in Karachi, Pakistan, and to screen the DLBCL patients for EZH2 mutation.
METHODOLOGY: A cross-sectional study was conducted on DLBCL patients, and IHC analysis, PCR, and sequencing of the EZH2 gene were used. This study was conducted from August 2016 to July 2020. Surgical specimens received in the histopathology section of DDRRL centrally and from different collection units throughout Sindh having been diagnosed with DLBCL were collected. Three markers (CD10, BCL6, and MUM1) were used for subtyping. DNA was manually extracted from the FFPE Blocks of DLBCL cases using phenol: chloroform: isopropanol alcohol. Polymerase chain reaction (PCR) was performed, and samples were sent from Dow University to Korea (Macrogen) for sequencing. The amplified product was 189bp. Primers were used for EZH2 to flank the region containing the tyrosine domain on exon 16 and exon 18.
RESULTS: Out of 116 cases, 60.3% and 39.7% were male and female, respectively. The mean age was 49.73±16.38 years. The mean survival duration of cases diagnosed with GCB was 8.21±7.39 months. Polymorphism of *EZH2* mutation (Tyr 701 Asn) was found to be positive in GCB-DLBCL for three samples (16.6%).
CONCLUSION: In the current study, Sanger sequencing was used to detect any mutation in the coding region of EZH2, and a unique mutation was reported (Tyr701Asn).

KEYWORDS: Germinal Center, Diffuse Large B Cell Lymphoma, Immunomarkers, EZH2 mutation, Polymerase chain reaction, sequencing

INTRODUCTION

Lymphomas are types of blood cancer originating from the lymphocytes, which develop in the cells of the lymph system[1]. The lymphomas are of two major types, Hodgkin (HL) and Non-Hodgkin (NHL)[2,3]. Globally, lymphomas are ranked between fifth and ninth among all malignancies[4]. Numerous previous researches have demonstrated that developing nations, such as Pakistan, have seen an increase in the overall prevalence of lymphomas[5-7]. The NHLs are divided into T-cell lymphomas and B-cell lymphomas[4]. Over the past 20 years, primary extranodal lymphoma incidence has increased about twice as much as nodal lymphomas[9]. According to the Shaukat Khanum Memorial Cancer Hospital (SKMH), the incidence and prevalence of NHL are 72.2%[10]. Among these, DLBCL is the most common cancer, which is approximately 42.6 % [3 (3)] and 35% [9 (9)] in our setup. DLBCL is classified based on gene expression profiling[11] and cell-of-origin (COO) of tumors from where they arise[12]. By classifying DLBCL into activated B-cells (ABC) and germinal centre B-cells (GCB), high throughput technologies like gene expression profiling (GEP) could help better comprehend the molecular diversity across physically comparable variants of DLBCL. Gene rearrangements in the immunoglobulin heavy and light chains are present in most DLBCL patients. B cell lymphoma 6 (BCL6) and B cell leukemia or lymphoma 2 (BCL2) proteins are expressed by about 70% and 80% of DLBCL, respectively[13]. GCB subtype originates from the germinal centre, has a better prognosis than the ABC type of DLBCL, and carries a different mutational spectrum[14]. Epigenetic factor-like histone modifications may enhance the pathogenesis of germinal centre DLBCL, such as in the case of EZH2[15]. EZH2 is an enzymatic component of PRC2 and encodes a histone methyltransferase. It has a role in the methylation of histone 3 on lysine 27 (H3K27me), resulting in transcriptional restriction or

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transcriptional repression. As EZH2 is necessary for a germinal centre, formation and its mutation have been associated with lymphoid transformation as it is overexpressed in many other cancers like ovarian, prostate, and breast cancer[16]. Upregulation of EZH2 is noticed in different cancers because it controls or maintains the function of those genes responsible for regulating the embryonic development of EZH2. It also inhibits the genes required to suppress tumor growth and development and is eventually related to poor prognosis. Hence, we can stop the tumorigenesis (growth production and promotion) by inhibiting or blocking the activity of methyltransferase[17]. Somatic mutation on the Y641 site was reported in GCB DLBCL (21.7%) and 7.2% in FL[16]. Heterozygous mutation of Y641 is the most commonly found EZH2 mutation mainly noticed in germinal centre DLBCL, which is approximately 22%[18] and A677G in roughly 1% to 2% of DLBCL, recurrent mutation of EZH2 A687 and A692 has been highlighted in several studies of non-Hodgkin lymphoma[14]. This recurrent mutation occurs on exon 15 of this gene, leading to this protein's structure change. Due to this unique association of Y641 and A677G with GCB, several therapeutic compounds (GSK126, EPZ005687, EPZ-6438) have been identified for treating GCB patients[16]. FL and the GCB type of DLBCL derive from the germinal centres of B-cells. Mutations in different genes involving the NFkB pathway picked by targeted re-sequencing studies[19] showed their presence in ABC type of DLBCL, but few GCB-specific mutations have also been identified[20]. In EZH2, point mutation and single nucleotide polymorphism are noted to a large extent, such as replacement of a single amino acid tyrosine in the SET domain of the EZH2 protein (Y641) occurs in 21.7% of GCB DLBCLs, 7.2% of FLs and are absent from ABC DLBCLs. A677G in roughly 1% to 2% of DLBCL. Recurrent EZH2 A687 and A692 mutations have been documented in various studies of non-Hodgkin lymphoma[14]. EZH2 proteins with mutated Y641 have decreased in vitro enzymatic activity. Advanced technology in DNA sequencing has recently permitted the characterization of genomes and transcriptomes at sufficient resolution for the identification of somatic point mutations[21].

The current study is a small attempt to identify mutations in EZH2 in lymphoma patients in the Dow Diagnostic Research and Reference Lab (DDRRL), Dow University of Health Sciences (DUHS), Karachi, Pakistan.

METHODOLOGY

A cross-sectional study was conducted on DLBCL patients, and IHC analysis, PCR, and sequencing of the EZH2 gene were used. This study was conducted from August 2016 to July 2020. Surgical specimens received in the histopathology section of DDRRL centrally and from different collection units throughout Sindh having been diagnosed with DLBCL were collected. 116 Diagnosed cases of DLBCL with consensus in diagnosis, patient consent and adequate tumor material were used for immunohistochemistry and DNA extraction. The relevant clinical data, such as age, gender, family history, procedure history, surgery, chemotherapy or radiotherapy, and survival rate required for the study, were collected through direct interviews with the patient or their attendants. The detailed gross description was noted. After sectioning and slide preparation of all selected cases, these were processed with routine H & E staining. Then, these sections were stained further with all the particular immunohistochemical makers, i.e. CD10, BCL6 & MUM1. Ki67, a tumor proliferative marker, was also scrutinized to evaluate the prognosis and survival status of DLBCL patients.

The study was approved (IRB-772/DUHS/ Approval/2016/296) by the Institutional Review Board of Dow University of Health Sciences.

Immunohistochemical staining

Immunohistochemistry was done by using a panel of CD10 (Dako), BCl6 (Dako,) and MUM-1. Then, paraffin-embedded tissues were cut into 3-4 µm thick sizes, and after that, tissues were deparaffinized, followed by dehydration in graded alcohol from 100%-50% too.

DNA extraction from FFPE & PCR

The tissues were then manually processed for DNA extraction from FFPE & PCR to analyze the expression of EZH2. Extraction and purification methods were performed via the following steps: To the dewaxed tissue, add 500 µl phenol: chloroform: isopropanol alcohol at 25:24:1. It is followed by vortex and centrifugation at RT, 12,000 × g for 10 min. A 1.5 ml microfuge tube was filled with supernatant fluid with the help of a 100-µl pipette; then, it was added with one volume of chloroform. After that, it was mixed for 5 mins by vortexing and centrifuged at 12,000. The Microfuge tube was filled with an aqueous supernatant, and then 0.1 volume of 3 M sodium acetate was added and mixed with vortexing, followed by 1 volume of isopropanol. Incubation was performed at −20 ºC overnight. The obtained DNA was centrifuged at 12,000 at 4 ºC. The yielded DNA was measured using the photometer technique as recommended by the manufacturer. The exon 18 of the EZH2 gene was amplified with primers forward, 5'-CCCTGAAGAACTGTAACCAG-3'; reverse, 5'-TTTCCAATCAAACCCACAGAC-3' as reported previously. The amplified product was run on 2% agarose.

Sequencing & Mutation Analysis

The successfully amplified PCR product was sent to Macrogen (Korea) for commercial DNA sequencing. The obtained sequences were trimmed and aligned with EZH2 reference sequences LGR_531 (NG_032043.1) using MEGA6 for mutation analysis. These sequences were further translated in-silico by the Expasy online tool to determine amino acid substitutions.

Statistical Analysis

The data was analyzed by using SPSS version 21. Mean and standard deviation were calculated for the quantitative variables and frequency and percentage of qualitative variables. Stratification was completed, and a post-stratification independent t-test was applied for mean comparison. The Chi-square test was put in to observe the influence of modifiers on the outcome. Survival analysis and a post hoc log-rank test were applied. Kaplan Mayer's test was used, and results show significance with mutation type (p=0.021) and procedure/ type of treatment done (chemotherapy, radiotherapy or a couple of treatments) (p=0.000). P-value ≤ 0.05 was considered as significant.

RESULTS

Out of 116 cases, 70.0(60.3%) were male and 46.0 (39.7%) were female. The mean age was 49.73 ([16.38%]) years. Mean Ki67, Mean CD10, Mean BCL6, and Mean MUM1 was 68.95(12.54%), 33.79 (23.91%), 33.79(17.67%), and 25.43(18.14%) respectively. Among 116 cases, 82.0(70.7%) were expired, while the Mean survival time for expired cases was 14.20(7.57) months. Chemotherapy was done for 72.0(11%), radiotherapy for 11.0(9.5%) and both chemotherapy and radiotherapy for 13.0(12%) while Surgery was done for 37.0(31.9%) cases. CD10 was found positive for 76.0(65.5%), negative for 38.0 (32.8%) and focally positive for 2.0(1.7%) cases, BCL6 was positive for 91.0(78.4%) and negative for 25.0(21.6%) while MUM1 was positive for 69.0 (59.5%), negative for 46.0(39.7%) and focally positive for 1.0(0.9%). In our study, 34.0(29.3%) cases were found with ABC and 82.0(70.7%) with GCB by immunohistochemistry (IHC) diagnoses. The total number of nodal vs. extra nodal cases was 69 and 47, respectively. In cases of nodal presentation, 32.7% were from the cervical region, 9.4% from the axilla, and 10.3% from the inguinal region, with 4.3% from the lymph node without a specific site identified. Lymph node sites showed no trends for specific diagnosis types (**Table I**).

Table I: Frequency of DLBCL cases with distribution of lymphoma in the nodal location

Gender	Frequency (%)
Male	70 (60.3)
Female	46 (39.7)
CD10	**Frequency (%)**
Positive	76 (65.5)
Negative	38(32.8)
Focal positive	2(1.7)
BCL6	**Frequency(%)**
Positive	91(78.4)
Negative	25(21.6)
MUM1	**Frequency(%)**
Positive	69(59.5)
Negative	46(39.7)
Focal Positive	1(0.9)
DISTRIBUTION OF LYMPHOMA IN NODAL LOCATION	
NODAL LOCATION n= 69 / 116 = 59.4%	
Neck / Cervical LN	38
Axillary LN	11
Inguinal / groin LN	12
LN, NOS	5
Supraclavicular LN	1
Submandibular LN	2
EXTRANODAL LOCATION n=47 /116 =40.6	
GIT n=31 /116	
Epigastrium	2
Stomach (gastric)	10
Ilieum& Caecum	4
Colon / Rectum	3
Tonsils	4
Liver	2
Spleen	2
Tongue	2
Palate	2
SOFT TISSUE 2/116	2
EXTREMITIES / BONE 3/116	
Bone	2
Shoulder	1
ABDOMEN 2/116	
Mesentery	1
Peritoneum	1
RESPIRATORY 5/116	
Nasal / Nasopharynx	4
Maxillary sinus	1
THORAX 1/116	
Chest wall	1
TESTES 3/116	3

Sequencing

Six samples (16.6) from 36 cases presented with EZH2 mutation in exon 18 of the Ezh2 gene; three were positive for an *EZH2* mutation (Tyr 701 Asn). No different nucleotide substitutions were seen in four cases. All cases were diagnosed as GCB-DLBCL on immunohistochemistry. No mutation was detected in exon 16 of the EZH2 gene. **Table II**

Sequence Analysis

The nucleotide sequence of EZH2 was aligned with the EZH2genomic reference sequence LGR_531 (NG_032043.1) using MEGA 6.0 software and the Clustal W algorithm method. Subsequently, the nucleotide sequences were translated into amino acid sequences by the ExPASY translated tool.

This figure shows mutational analysis of the EZH2 gene. Mutational analysis was done through MEGA

6.0. We found a substitution of A with G at nucleotide number 80015 in sample 12, T with A at nucleotide number 80031 in samples 11,16 and 17, G with A at nucleotide number 80060 in sample 12, A with C at nucleotide number 80079 in sample 15.

Table II: Detail of the SNV observed

S.#	Sample ID	Genomic Mutation	A.A Change
1	13-15776	LGR_531:g.79957**dupT (*)**	
2	13-13018	LGR_531:g.80015**G>A (Heterozygous)**	
3	13-13018	LGR_531:g.80060**G>A**	
4	15-15327	LGR_531:g.80031**T>A (Heterozygous)**	p.Tyr701Asn
5	15-12842	LGR_531:g.80031**T>A (Heterozygous)**	p.Tyr701Asn
6	15-22655	LGR_531:g.80031**T>A (Heterozygous)**	p.Tyr701Asn
7	15-21969	LGR_531:g.80079**A>C**	

Nucleotide Numbering is based on the EZH2 genomic reference sequence LGR_531 (NG_032043.1).
Changes in protein are predicted by Mutalyzer 2.0.26 (https://mutalyzer.nl/)(*)The duplication of T on LGR_531:g.79957 is near the slice site but not in the exon

Figure I: Mutational analysis of EZH2 gene

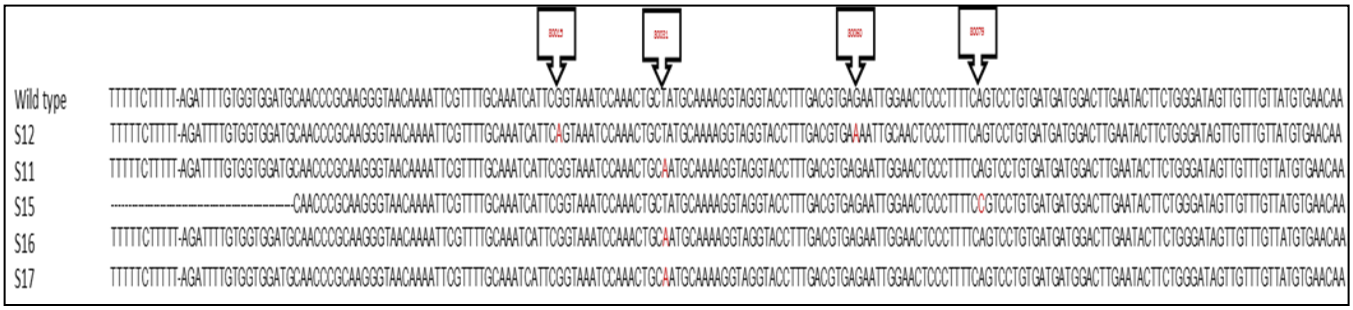

Figure II: Translational of amino acid sequence by using expasy

This figure shows mutational analysis of the EZH2 gene. First, nucleotide sequences were translated into amino acid sequences by using ExPASY. Mutational analysis was done through MEGA 6.0. We found a substitution of asparagine, denoted by (N), in place of tyrosine, denoted by (Y) at position 701 of EZH2.

DISCUSSION

Several somatic mutations have been detected in DLBCL patients, but their functional consequence still needs to be resolved. In the current study, Sanger sequencing was used to detect any mutation in the coding region of EZH2 and report a unique mutation (Tyr701Asn). Compared to the current study, whole-exome sequencing was used to analyze any germ line variations. The large-scale sequencing of cancer genomes necessitates an unbiased reevaluation of the mutations that lead to cancer. One study noticed a mutation on exon 15 of the *EZH2* gene, which encodes a portion of the EZH2, as compared to our research, which shows that out of 36 samples that we have sequenced, 16.6% (n=6) were found to be mutated in exon 18 of Ezh2 gene. The very known gene expression configuration of GCB-DLBCL is the same as that of normal GC-B-cells[22].

Additionally, these tumors hold classic features of normal B-cells, such as class switch recombination (CSR) and somatic hypermutation (SHM)[23]. Multiple chromatin-modifying genes are documented for mutation in DLBCL, with occurrence in GCB-DLBCL and repeated somatic mutations that alter a single residue in the Polycomb-group oncogene EZH2 found in 21.7% of GCB type of DLBCL. EZH2 is a complying part of Polycomb Repressive Complex trimethylate Lys of histone H3[24], increasing its affinity for the substrate[16]. Gain-of-function mutations occur in 22% of patients with GCB-DLBCL, substituting single evolutionarily preserved tyrosine residue (Tyr641), resulting in higher methylation levels in vitro owing to altered substrate selectivity[25]; this shows a remarkable survival difference in the subgroups mentioned above of patients with an overall survival rate at five years of 76% for GCB and 16% for ABC DLBCL patients. Unlike our study, in which the mortality rate was 64.7% in cases diagnosed with ABC and 73.2% in cases diagnosed with GCB. EZH2 mutation detected in DLBCL is characterized as encouraging new therapeutic targets as suggested by different published data. Commercially available compounds that inhibit the pathogenesis of EZH2 provoke cell cycle arrest and apoptosis expressly in EZH2-mutated DLBCL cells[26].

CONCLUSION

Despite its heterogeneity, DLBCL can be distinguished by its primary hereditary lesions and associated pathways that may be targeted by

personalized medication. Several unidentified genes and diverse mechanisms influence the pathogenesis of DLBCL. Histone modifications epigenetic factors could potentially worsen the pathophysiology of germinal centre DLBCL, as EZH2 does. These mutations can inaugurate new therapeutic options or encourage established therapies.

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AUTHOR CONTRIBUTION

Abbas FF: Principal Investigator, Design research study, concept making, Project administration
Bari MF: Conceptualization, Design methodology and supervised the findings of this work
Mughal MN: Critically reviewed the manuscript, to investigate the cases, Implementation of critical experimental protocols, C0-Supervised the findings of this work
Khan BA: Data analysis, data interpretation especially molecular and bioinformatics of research work
Khan N: Acquisition of Data, statistical analysis, verified the analytical methods
Kamil S: Data collection, Implementation of critical experimental protocols
Razzaq A: Data collection, , data interpretation especially histopathological laboratory work
Kamil N: Implementation of critical experimental protocols, Resolved the problems of accuracy or integrity of the work, data interpretation for the work

REFERENCES

1. Piñeros M, Laversanne M, Barrios E, de Camargo Cancela M, de Vries E, Pardo C et al. An updated profile of the cancer burden, patterns and trends in Latin America and the Caribbean. Lancet Reg Health-Am. 2022 Sep; 13: 100294. doi: 10.1016/j.lana.2022.100294.
2. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T et al. Malignant lymphomas in Pakistan according to the WHO classification of lymphoid neoplasms. Asian Pac J Cancer Prev. 2008; 9(2): 229-32.
3. Lazim NM, Abdullah B. Multidisciplinary approach to children with sinonasal tumors: A review. Pediatr Investig. 2019; 3(3): 173-9. doi: 10.1002/ped4.12147.
4. Miranda-Filho A, Piñeros M, Znaor A, Marcos-Gragera R, Steliarova-Foucher E, Bray F. Global patterns and trends in the incidence of non-Hodgkin lymphoma. Cancer Causes Control. 2019; 30(5): 489-99. doi: 10.1007/s10552-019-01155-5.
5. Khorshid O, Salama A, Helal A, Sallam Y, Amin M. Clinicopathologic profile and outcome of extranodal diffuse large B-cell NHL Egyptian National Cancer Institute Experience. Forum Clin Oncol. 2015; 6(3):8-12. doi: 10.1515/fco-2015-0013.
6. Menon MP, Pittaluga S, Jaffe ES. The histological and biological spectrum of diffuse large B-cell lymphoma in the World Health Organization classification. Cancer J. 2012; 18(5): 411-420. doi: 10.1097/PPO.0b013e31826aee97.
7. Alyahya N, Adiga B, Alwadei A, Alshahrani G, Alyahya F. The clinicopathological profile of non-Hodgkin's lymphoma in Aseer region of Saudi Arabia. BMC Res Notes. 2019; 12(1): 418. doi: 10.1186/s13104-019-4447-1.
8. Diumenjo MC, Abriata G, Forman D, Sierra MS. The burden of non-Hodgkin lymphoma in Central and South America. Cancer Epidemiol. 2016; 44: S168-S177. doi: 10.1016/j.canep. 2016.05.008.
9. Mahmood H, Habib M, Aslam W, Khursheed S, Fatima S, Aziz S et al. Clinicopathological spectrum of Diffuse Large B Cell lymphoma: a study targeting population yet unexplored in Pakistan. BMC Res Notes. 2021; 14: 1-6. doi: 10.1186/s13104-021-05768-5.
10. Ali S, Ali M, Badar F, Basit A, Hameed A. Factors Associated with Increased Red Blood Cells Transfusion Requirements in Patients with Hodgkin and Non-Hodgkin Lymphoma. J Ayub Med Coll Abbottabad. 2015; 27(1): 70-3.
11. Padala SA, Kallam A. Diffuse large B cell lymphoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan, 2023 Apr 24.
12. Johnson N, Assouline S, Alcaide M, Mohajeri A, Greenwood C, Riazalhosseini Y et al. Genetic landscapes of relapsed and refractory diffuse large B cell lymphomas. Cancer Res. 2016; 76 (14):117. doi: 10.1158/1538-7445.AM2016-117.
13. Colomo L, López-Guillermo A, Perales M, Rives S, Martínez A, Bosch F et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood. 2003; 101(1): 78-84. doi: 10.1182/blood-2002-04-1286. Epub 2002 Aug 8.
14. Chapman JR, Bouska AC, Zhang W, Alderuccio JP, Lossos IS, Rimsza LM et al. EBV-positive HIV -associated diffuse large B cell lymphomas are

characterized by JAK/STAT (STAT3) pathway mutations and unique clinicopathologic features. Br J Haematol. 2021; 194(5): 870-878. doi: 10.1111/bjh.17708. Epub 2021 Jul 17.
15. Li SS, Zhai XH, Liu HL, Liu TZ, Cao TY, Chen DM et al. Whole-exome sequencing analysis identifies distinct mutational profile and novel prognostic biomarkers in primary gastrointestinal diffuse large B-cell lymphoma. Exp Hematol Oncol. 2022; 11(1): 71. doi: 10.1186/s40164-022-00325-7.
16. Narazaki T, Nakashima Y, Tsukamoto Y, Tsuda M, Masuda T, Kimura D et al. Transformation of follicular lymphoma to double-hit lymphoma during adjuvant chemotherapy for concurrent ovarian carcinoma. Int J Hematol. 2019; 110(3): 375-80. doi: 10.1007/s12185-019-02656-4. Epub 2019 May 18.
17. Erokhin M, Chetverina O, Győrffy B, Tatarskiy VV, Mogila V, Shtil AA et al. Clinical correlations of polycomb repressive complex 2 in different tumor types. Cancers(Basel). 2021; 13(13): 3155. doi: 10.3390/cancers13133155.
18. Li B, Chng WJ. EZH2 abnormalities in lymphoid malignancies: underlying mechanisms and therapeutic implications. J Hematol Oncol. 2019; 12(1): 118. doi: 10.1186/s13045-019-0814-6.
19. Dharanipragada P, Parekh N. Genome-wide characterization of copy number variations in diffuse large B-cell lymphoma with implications in targeted therapy. Precis Clin Med. 2019; 2(4): 246 -58. doi: 10.1093/pcmedi/pbz024. Epub 2019 Nov 21.
20. Morin R, Bainbridge M, Fejes A, Hirst M, Krzywinski M, Pugh T et al. Profiling the HeLa S3 transcriptome using randomly primed cDNA and massively parallel short-read sequencing. Biotechniques. 2008; 45(1): 81-94. doi: 10.2144/ 000112900.
21. Chen G, Shi T, Shi L. Characterizing and annotating the genome using RNA-seq data. Sci China Life Sci. 2017; 60(2): 116-125. doi: 10.1007/s11427-015-0349-4. Epub 2016 Jun 13.
22. Montesinos-Rongen M, Brunn A, Sanchez-Ruiz M, Küppers R, Siebert R, Deckert M. Impact of a Faulty Germinal Center Reaction on the Pathogenesis of Primary Diffuse Large B Cell Lymphoma of the Central Nervous System. Cancers(Basel). 2021; 13(24): 6334. doi: 10.3390/ cancers13246334.
23. Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A. 2010; 107 (49): 20980-5. doi: 10.1073/pnas.1012525107. Epub 2010 Nov 15.
24. Lee EF, Fairlie WD. Discovery, development and application of drugs targeting BCL-2 pro-survival proteins in cancer. Biochem Soc Trans. 2021; 49 (5): 2381-95. doi: 10.1042/BST20210749.
25. Merdan S, Subramanian K, Ayer T, Van Weyenbergh J, Chang A, Koff JL et al. Gene expression profiling-based risk prediction and profiles of immune infiltration in diffuse large B-cell lymphoma. Blood Cancer J. 2021; 11(1): 2. doi: 10.1038/s41408-020-00404-0.
26. Zamò A, Gerhard-Hartmann E, Ott G, Anagnostopoulos I, Scott DW, Rosenwald A et al. Routine application of the Lymph2Cx assay for the subclassification of aggressive B-cell lymphoma: report of a prospective real-world series. Virchows Arch. 2022; 481(6): 935-943. doi: 10.1007/s00428-022-03420-6. Epub 2022 Oct 11.