Comparison of tumor mutation burden of 300 various non-Hodgkin lymphomas using panel based massively parallel sequencing

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Abstract

Background: Tumor mutation burden is an emerging biomarker for immunotherapy. Although several clinical trials for immunotherapy in lymphoma have been carried out, the mutation burden of various lymphomas is not well known yet. Thus, the objective of this study was to compare tumor mutation burden of various non-Hodgkin lymphomas using panel based massively parallel sequencing.

Methods: We conducted 405 gene panel based massively parallel sequencing of 300 non-Hodgkin lymphomas and investigate the number of SNV/Indel in each lymphoma.

Results: The number of SNV/Indel was higher in mature B-cell lymphoma than in mature T- and NK-cell lymphoma.($P < 0.001$) The number of SNV/Indel in primary mediastinal large B-cell lymphoma and primary diffuse large B-cell lymphoma of the central nervous system was the highest, which was significantly higher than that in diffuse large B-cell lymphoma, not otherwise specified (DLBCL NOS).($P = 0.030$ and $P = 0.008$, respectively) The SNV/Indel number in EBV-positive DLBCL NOS was significantly lower than that in DLBLC NOS.($P = 0.048$) Peripheral T-cell lymphoma, NOS showed no significant difference in the number of SNV/Indel from extranodal NK/T-cell lymphoma, nasal type ($P = 0.942$) or angioimmunoblastic T-cell lymphoma ($P = 0.739$). The number of SNV/Indel in anaplastic large cell lymphoma, ALK-positive was significantly lower than that in anaplastic large cell lymphoma, ALK-negative ($P = 0.049$). It showed the lowest SNV/Indel number among all lymphomas.

Conclusion: Various lymphomas have different mutation burdens. Thus, tumor mutation burden could be used as a promising biomarker for immunotherapy in lymphomas.

Background

Tumor mutation burden (TMB) is one of the most valuable biomarkers to identify patients who are likely to respond to immune checkpoint blockade [1-4]. Tumor cells harboring more mutations have a higher chance of producing neoantigens that are recognized and targeted by the host immune system [5-9]. Host immune cells can be soldiers that kill cancer cells and immune checkpoint blockades can upregulate anti-tumor activity of host immune cells, such as cytotoxic T-cells [10-12].

TMB is not only a biomarker to predict response to immunotherapy, but also has several other meanings. The number of mutations varies across tumor types. It can reflect different mutational signature and tumorigenesis of each malignancy [1, 13-16]. For example, cutaneous squamous cell carcinomas have higher TMB, while uterine cervix squamous cell carcinomas have lower TMB [13]. This difference is due to the fact that etiologies of these two squamous cell carcinomas are different, i.e., ultraviolet light for skin cancer and human papillomavirus infection for uterine cervix cancer. Moreover, the number of mutations can reflect the progress of neoplasms. In general, if the neoplasm progresses, mutations are likely to accumulate. This is not only due to the instability of DNA, but also due to tumor evolution for evading immune surveillance and for cancer survival [17-19]. In this respect, comparing the number of mutations between different tumors and within the same tumor group can provide interesting information about the tumor.

Immunotherapy has been emerging as an important treatment modality not only in carcinomas and melanomas, but also in lymphoid malignancies [20, 21]. So far, studies on immunotherapy for lymphoid malignancy have been mainly conducted for classic Hodgkin lymphoma [22-24]. However, effects of immunotherapy for a subset of non-Hodgkin lymphomas have been reported [25, 26]. Although the significance of TMB in lymphomas is increasing, studies on TMB in lymphomas are insufficient. Thus, the objective of this study was to investigate the number of single nucleotide variant (SNV) and Indel of 300 various non-Hodgkin lymphomas using massively parallel sequencing with a 405-gene panel.

Methods

Patient selection

Patients diagnosed with lymphoma in Samsung medical center (Seoul, Korea) are enrolled in the ‘SMC lymphoma registry’ with informed consent. From January 2019, next generation sequencing (NGS) has been performed on the patients with sufficient tumor sample volumes both in patients with the first diagnosis and relapsed/refractory patients. In this study, results of 300 non-Hodgkin

lymphoma patients who underwent NGS by December 2020 were analyzed. The pathologic diagnosis was made according to 2016 WHO classification [27] by two pathologists (JC and YHK).

Panel Based Massively Parallel Sequencing (Lymphomascan)

Targeted genetic sequencing was performed using LymphomaSCAN panel, including whole exomes of 405 genes related to hematological malignancies (Supplementary Table 1). Extracted genomic DNA was sheared using a Covaris S220 (Covaris, Woburn, MA, USA). Targeted gene was captured using a SureSelect XT Reagent Kit, HSQ (Agilent Technologies) and a paired-end sequencing library was constructed with a barcode. DNA sequencing was performed on a NextSeq 550 Dx sequencer (Illumina, San Diego, CA, USA). The paired-end reads were aligned to the human reference genome (hg19) using BWA-MEM v0.7.5, Samtools v0.1.18, GATK v3.1-1, and Picard v1.93. We called single-nucleotide variants using MuTect version 1.1.4, Lowfreq version 0.6.1, and VarDict version 1.06 software with a variant allele frequency ≥ 1% or the number of variant supporting reads > 4. We filtered out sequencing errors using a machine learning algorithm with features extracted from SAM files. By this algorithm, we could increase the specificity of results [28]. We manually reviewed variants with supporting reads < 20 using an Integrative Genomics Viewer browser and filtered out sequencing errors. We identified small insertions and deletions using Pindel version 0.2.5a4 with the number of variant supporting reads > 9. We further filtered out variants present with a minor allele frequency ≥ 1% in the 1000 Genomes Project database (http://www.internationalgenome.org/), the Exome Aggregation Consortium database (http://exac.broadinstitute.org/), the National Heart, Lung, and Blood Institute's Exome Sequencing Project database (https://esp.gs.washington.edu/drupal/), the Korean Reference Genome Database (http://152.99.75.168/KRGDB/), the Korean Variant Archive (https://kobic.re.kr/kova/), and an in-house database from 192 Korean individuals. We selected missense variants predicted to have a functional consequence by Mendelian Clinically Applicable Pathogenicity score [29] with the author's recommended threshold. To measure the number of mutation consistently, only SNV/indel results were used while copy number variation and fusion results were discarded. To filter out false-positive results, variants with variant allele frequency (VAF) less than 5% and total reads less than 100 were excluded.

Statistical analysis

We used the SPSS 27 statistical software program (IBM Corporation) for all statistical analyses. Mann–Whitney U test was performed to test the difference between two tumors of TMB. P values < 0.05 were considered statistically significant.

Results

Patients' characteristics

The median age of 300 patients was 58 years (range, 19–90 years). There were 187 males and 113 females (male to female ratio, 1.65:1).(Table 1) Among 300 lymphomas, there were 243 (81.0%) mature B-cell neoplasms, 53 (17.6%) mature T- and NK-cell neoplasms, and 4 (1.3%) precursor lymphoid neoplasms. In mature B-cell neoplasms, diffuse large B-cell lymphoma, not otherwise specified (DLBCL NOS) was the most common with 154 patients, followed by follicular lymphoma in 29 patients, primary diffuse large B-cell lymphoma of the central nervous system (CNS DLBCL) in 17 patients, and mantle cell lymphoma in 11 patients. Among mature T- and NK-cell neoplasms, extranodal NK/T-cell lymphoma, nasal type (ENKTL) was the most common (18 patients), followed by peripheral T-cell lymphoma, not otherwise specified (PTCL NOS) and angioimmunoblastic T-cell lymphoma (AITL) (10 patients each).

Number of SNV/Indel in all cases

When the number of SNV/Indel was counted by dividing VAF by 1%, VAF between 39% and 52% was higher than expected.(Fig. 1) Therefore, this area was considered to be a section with a high probability of including germline mutations. Excluding VAF less than 5% and total read less than 100, the average of the number of SNV/Indel was 22.68 (6804/300). The average number of SNV/Indel was 23.98 in mature B-cell neoplasms and 17.21 in mature T- and NK-cell neoplasms, showing a significant ($P < 0.001$) difference between the two. When all lymphomas were arranged in the order of median value of the number of SNV/Indel mutations, primary mediastinal large B-cell lymphoma (PMLBL) (median: 32) was the highest, followed by CNS DLBCL (median: 30), DLBCL NOS (median: 23), and anaplastic large cell lymphoma, ALK-negative (ALCL, ALK-) (median: 23).(Fig. 2) Lymphomas with the lowest SNV/Indel number were in order of ALCL, ALK-positive (ALCL, ALK+) (median: 14), follicular T-cell lymphoma (median: 14), and nodal peripheral T-cell lymphoma with TFH phenotype (PTCL TFH) (median: 14.5). The number of variants corresponding to the VAF 39–52% section did not show significant difference according to the type of lymphoma.($P = 0.529$) (Fig. 2B)

Number of SNV/Indel in diffuse large B-cell lymphoma variants

Regarding types of DLBCL NOS according to Hans' classification [30], 93 cases had a post-germinal center B-cell type (activated B-cell type, ABC type) and 53 cases had a germinal center B-cell type (GCB type), showing no significant difference in number of SNV/Indel between these two types of DLBCL NOS.(P = 0.308) (Fig. 3A) Compared with DLBCL NOS, PMLBL (P = 0.030) and CNS DLBCL (P = 0.008) had more SNV/Indel mutations, while EBV-positive diffuse large B-cell lymphoma, not otherwise specified (EBV DLBCL) had significantly (P = 0.048) less SNV/Indel mutations. High grade B-cell lymphoma (HGBCL) (P = 0.287), including double-hit lymphoma and triple-hit lymphoma and DLBCL NOS admixed with extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), showed no significant (P = 0.199) difference from DLBCL NOS in SNV/indel number. There was no significant difference of SNV/Indel number according to Ann-Arbor stage (Fig. 3B) and therapeutic status (Fig. 3C) in DLBCL variants.

DLBCL, diffuse large B-cell lymphoma; CNS, central nervous system; NOS, not otherwise specified, GCB, germinal center B-cell; ABC, activated B-cell; MALT, mucosa-associated lymphoid tissue, EBV, Epstein-Barr virus; PMLBL, primary mediastinal large B-cell lymphoma, HGBCL, high-grade B-cell lymphoma.

Number of SNV/Indel in mature B-cell lymphomas except DLBCL variants

Results of comparison of the number of SNV/Indel of mature B-cell lymphoma except DLBCL variants are depicted in Fig. 4A. When follicular lymphoma was classified into low grade (grade 1–2, n = 19) and high grade (grade 3A and 3B, n = 10) by histologic grading [27], the number of SNV/indel of high grade follicular lymphoma (median: 20) was significantly (P = 0.013) higher than that of low grade (median: 17). Mantle cell lymphoma, lymphoplasmacytic lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and nodal marginal zone lymphoma showed no significant difference in the number of SNV/Indel. When compared with DLBCL NOS, low grade follicular lymphoma (P < 0.001) and mantle cell lymphoma (P = 0.026) had significantly less SNV/Indel numbers while high grade follicular lymphoma showed no significant difference (P = 0.973). There was no significant difference of SNV/Indel number according to Ann-Arbor stage (Fig. 4B) and therapeutic status (Fig. 4C) in mature B-cell lymphomas. The remaining small B-cell lymphomas were not suitable for statistical analysis due to small number of cases. However, all of them showed lower SNV/Indel median values than mantle cell lymphoma.

MALT, mucosa-associated lymphoid tissue; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; MALToma, extranodal marginal zone lymphoma of MALT; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; NMZL, nodal marginal zone lymphoma.

Number of SNV/Indel in mature T- and NK-cell neoplasms

PTCL NOS showed no significant difference in SNV/Indel number from ENKTL (P = 0.942) or AITL (P = 0.739).(Fig. 5A) When these three T-cell lymphomas of T follicular helper (TFH) cell origin were compared, the median number of SNV/Indel of AITL was higher than that of follicular T-cell lymphoma (P = 0.133) or PTCL TFH (P = 0.056), although the difference was not statistically significant. In ALCL, the mutational burden was significantly higher in ALK-negative than in ALK-positive (P = 0.049). PTCL NOS showed significantly lower SNV/Indel than DLBCL NOS (P = 0.008). There was no significant difference of SNV/Indel number according to Ann-Arbor stage (Fig. 5B) and therapeutic status (Fig. 5C) in mature T- and NK-cell lymphomas. Tendencies to show more mutations in advanced disease (Ann-Arbor stage III or IV) or post-chemotherapy patients (relapsed/refractory) were observed in ENKTL, but there were not statistical significance.

PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; TFH, T follicular helper cell; ALCL, anaplastic large cell lymphoma, ALK, anaplastic lymphoma kinase; ENKTL, extranodal NK/T-cell lymphoma, nasal type; AITL, angioimmunoblastic T-cell lymphoma; FTCL, follicular T-cell lymphoma.

Table 1
Clinical characteristics and the number of SNV/Indel by pathologic diagnosis.

Diagnosis	Number of patients	Age median (range)	M:F	Ann-Arbor stage		Therapeutic status		Number of SNV/Indel		
				I or II	III or IV	Pre-Tx. (at diagnosis)	Post-Tx. (relapsed/ refractory)	Mean	Median	Range
Mature B-cell neoplasms	**243**									
Diffuse large B-cell lymphoma, NOS	154	61 (26–86)	93:61	71	83	125	29	24.84	23	11–87
Primary diffuse large B-cell lymphoma of the CNS	17	62 (34–86)	10:7	17	0	16	1	31.53	30	17–51
Primary mediastinal large B-cell lymphoma	5	36 (25–62)	3:2	1	4	5	0	31.80	32	26–41
EBV-positive diffuse large B-cell lymphoma, NOS	6	62 (19–90)	4:2	2	4	5	1	17.00	18	5–28
High-grade B-cell lymphoma	6	50.5 (37–63)	5:1	1	5	4	2	20.83	21.5	15–27
Burkitt lymphoma	1	69	0:1	0	1	0	1	22.00	22	
Plasmablastic lymphoma	2	58 (52–64)	2:0	1	1	1	1	19.50	19.5	17–22
Follicular lymphoma	29	50 (28–79)	18:11	5	24	27	2	19.62	18	8–35
Mantle cell lymphoma	11	63 (47–80)	8:3	2	9	7	4	19.09	20	11–27
Nodal marginal zone lymphoma	3	59 (58–63)	1:2	2	1	2	1	23.33	16	12–42
Extranodal marginal zone lymphoma of MALT	4	55.5 (44–68)	2:2	3	1	3	1	19.25	20.5	13–23
Lymphoplasmacytic lymphoma	2	59.5 (53–66)	2:0	0	2	0	2	17.00	17	14–20
Chronic lymphocytic leukemia/Small lymphocytic lymphoma	3	56 (53–61)	1:2	0	3	3	0	15.33	16	10–20
Mature T- and NK-cell neoplasms	**53**									
Peripheral T-cell lymphoma, NOS	10	48 (25–71)	7:3	3	7	5	5	17.60	18	11–23

SNV, single nucleotide variant; NOS, not otherwise specified.

Diagnosis	Number of patients	Age median (range)	M:F	Ann-Arbor stage		Therapeutic status		Number of SNV/Indel		
				I or II	III or IV	Pre-Tx. (at diagnosis)	Post-Tx. (relapsed/ refractory)	Mean	Median	Range
Angioimmunoblastaic T-cell lymphoma	10	66.5 (43–81)	5:5	0	10	7	3	18.70	19.5	10–24
Follicular T-cell lymphoma	2	50 (48–52)	2:0	0	2	1	1	14.00	14	13–15
Nodal peripheral T-cell lymphoma with TFH phenotype	4	68.5 (64–75)	3:1	0	4	3	1	14.25	14.5	13–15
Extranodal NK/T-cell lymphoma, nasal type	18	56 (32–79)	11:7	11	7	12	6	17.44	17	9–25
Anaplastaic large cell lymphoms, ALK-positive	5	35 (20–58)	4:1	1	4	4	1	12.20	14	6–16
Anaplastaic large cell lymphoms, ALK-neative	4	40 (29–56)	3:1	1	3	4	0	22.25	23	15–28
Precursor lymphoid neoplasms	**4**									
Lymphoblastic leukemia/lymphoma	4	44.5 (34–70)	3:1	1	3	4	0	19.00	19.5	15–22
Total	**300**	**58 (19–90)**	**187:113**	**122**	**178**	**238**	**62**	**22.68**	**21**	**5–87**

SNV, single nucleotide variant; NOS, not otherwise specified.

Discussion

In this study, we tried to find out whether there are differences in TMB of various non-Hodgkin lymphomas across B- and T-/NK-cell lymphomas. Whole Exome Sequencing (WES) is a gold standard to measure TMB in cancers. However, because of its high cost, WES is usually performed for research, not for diagnosis. Recently, several studies have shown that using a well-designed gene panel can calculate TMB which is very similar to that from WES [13, 31–34]. The gene panel used in our study consists of more than 400 genes designed for lymphomas, although the TMB calculated using them has not been validated through parallel WES. Rather than classifying TMB-high and TMB-low groups of lymphomas through precise cutoff value, we tried to compare the number of genetic mutations of various non-Hodgkin lymphomas under the same platform. And we were able to make some meaningful discoveries.

Overall, B-cell lymphomas had more mutations than T-cell lymphomas. This result is consistent with the existing knowledge that B-lymphocytes have a complex maturation process such as somatic hypermutation that T-lymphocytes do not have [35]. Among DLBCL variants, PMLBL and CNS DLBCL had more mutations than DLBCL NOS. Of all lymphomas included in this study, these two tumors were the only two lymphomas with median number of SNV/indel of 30 or higher. PMLBL is a distinct subtype from other DLBCL variants, and is known to have a gene expression profile that overlaps with classic Hodgkin lymphoma [36, 37]. Some studies have shown that a part of refractory and recurrent PMLBL showed responses to pembrolizumab, a programmed death-1 (PD-1)

blocker [26, 38]. This is thought to be related to the fact that frequent amplification and translocation events occur at 9p24.1, in which *CD274* (PD-L1) gene is located, in PMLBL [39, 40]. In addition, the formation of immune cell-rich microenvironment, such as classic Hodgkin lymphoma, is also a necessary condition for the action of immune checkpoint blockade. The high TMB observed in PMLBL may also be one of reasons why this tumor is eligible for immune checkpoint blockade treatment. CNS DLBCL also had a large number of SNV/Indels comparable to PMLBL. According to gene expression profiling analysis of CNS DLBCL, it was not markedly different from systemic DLBCL [41], although it mainly belongs to the post-germinal center B-cell type. Although not included in this study, in our NGS results, CNS DLBCL had a higher 9p21 (including *CDKN2A* and *CDKN2B*) loss ratio (13/17, 76.5%) than DLBCL NOS (43/154, 27.9%). In our cohort, CNS DLBCL is considered to be a group with specific features such as high TMB and 9p21 loss while it is genetically included in the spectrum of systemic DLBCL. Due to the specificity of immune sanctuary site, further studies are needed on the influence of high TMB of CNS DLBCL on the effectiveness of immune checkpoint blockades. It has been reported that CNS DLBCL also shows reactivity to Pembrolizumab [42]. On the contrary, EBV DLBCL showed significantly lower TMB than DLBCL NOS. This supports that EBV infection is a strong driver of tumorigenesis in B-cell lymphoma. In general, TMB-low cancer is considered to be less suitable for immunotherapy. However, apart from the number of mutations, EBV infection can generate neoantigens that can be targets of host immune cells [43]. Therefore, EBV DLBCL patients should not be excluded from candidates for immunotherapy, although they have a low TMB [44].

Mature B-cell lymphomas not included in DLBCL variants had lower TMB than DLBCL NOS, and the difference in SNV/Indel between them was not significant. The fact that the SNV/Indel of grade 3 follicular lymphoma was significantly higher than that of grade 1–2 follicular lymphoma suggests that the histologic grade of follicular lymphoma might go up with disease progression. The mutation burden of high-grade follicular lymphoma was between low grade follicular lymphoma and DLBCL NOS. Due to ethnic characteristics of our cohort, the number of CLL/SLL patients was only three. Besides CLL/SLL, small B-cell lymphomas in our study had a small number of samples. Thus, further studies using a sufficient number of cases are needed.

Several interesting findings were observed regarding the mutation burden in mature T cell lymphomas. There was no difference in mutation burden between PTCL NOS and ENTKL. This is in contrast with significantly lower mutation burden of EBV DLBCL compared to DLBCL NOS in B-cell lymphoma. Although ENKTL is not EBV-positive PTCL *per se*, it suggests that the role of EBV infection in the tumorigenesis of B-cell lymphoma and T-/NK-cell lymphoma is different [45, 46]. Among PTCLs derived from T follicular helper cell, mutation burden of AITL tended to be higher than the other two (follicular T-cell lymphoma and PTCL TFH), although the difference was not statistically significant. Considering that follicular T-cell lymphoma and PTCL TFH have similar molecular signatures to AITL [47], it supports the hypothesis that AITL is a more progressed disease than the other two lymphomas. It is known that ALK-positive and ALK-negative ALCLs share a common molecular signature [48], however, significantly higher mutation burden of ALCL ALK- than that of ALCL ALK + suggests that *ALK* gene translocation is a very strong oncogenic event. In addition, it is consistent with the fact that ALCL ALK- generally has poorer clinical outcome than ALCL ALK+, despite their similar histologic morphology [49].

Interestingly, number of SNV/Indel was not associated with Ann-Arbor stage in most lymphomas. High-grade lymphoma generally had more mutations than in low-grade lymphoma in our study, but there was no difference in number of mutations between advanced/systemic disease and localized disease in the same diagnosis. These findings suggest that high- or low-grade lymphomas develop by separate pathways, but accumulation of mutations is not a major mechanism of disease progression in most lymphomas. Moreover, history of chemotherapy was also not associated with an increase in the number of SNV/Indel. Although the diagnosis of patients in our cohort varies, most patients with relapsed/refractory tumor received CHOP-containing regimen as the first-line chemotherapy. Cyclophosphamide and doxorubicin are known as agents that damage DNA, but they did not actually increase the number of mutations in post-therapeutic lymphoma patients. Further research is needed on what these observations mean.

Our study has several limitations. First, we used panel-based target sequencing rather than WES. Although targeted sequencing has been widely used as a method for measuring TMB in various malignancies [31–34], and more than 400 genes were included in our panel, it has not been validated for TMB measurement. For this reason, our study did not suggest cutoff value for classifying TMB-high and TMB-low group lymphomas. Second, matched normal tissue was not used in our sequencing process. Thus, germline mutations were not clearly filtered out. Third, copy number variation and translocation results other than SNV/Indel were not enrolled in our analysis. Last, our study only analyzed the number of SNV/Indel, not specific mutant genes. It is expected that through further

studies that complement the above-described limitations, the TMB of lymphomas can be more accurately analyzed and the TMB-high group lymphoma eligible for immunotherapy can be classified.

Conclusion

In conclusion, different types of lymphoma have different numbers of mutations. The number of mutations might reflect clinical and pathologic characteristics of each lymphoma. TMB is expected to be used as a useful biomarker in the immunotherapy of various lymphomas.

List Of Abbreviations

TMB, tumor mutation burden; SNV, single nucleotide variant; NGS, next generation sequencing; VAF, variant allele frequency; DLBCL NOS, diffuse large B-cell lymphoma, not otherwise specified; CNS DLBCL, primary diffuse large B-cell lymphoma of the central nervous system; ENKTL, extranodal NK/T-cell lymphoma, nasal type; PTCL NOS, peripheral T-cell lymphoma, not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; PMLBL, primary mediastinal large B-cell lymphoma; ALCL, anaplastic large cell lymphoma, PTCL TFH, nodal peripheral T-cell lymphoma with TFH phenotype; ABC, activated B-cell; GCB, germinal center B-cell, EBV DLBCL, EBV-positive diffuse large B-cell lymphoma, not otherwise specified; HGBCL, High grade B-cell lymphoma; MALT lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; WES, whole exome sequencing

Declarations

Ethic approval and consent to participate

All methods were carried out in accordance with Helsinki declaration, and all protocols of this study were approved by the Institutional Review Board of Samsung Medical Center (IRB file number: SMC 2021-02-065).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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None

Authors' contributions

J.C. contributed to study design, data interpretation, data analysis, created the figures and led the write-up. S.E.Y. contributed to data interpretation, and data analysis. S.J.K. contributed to the model evaluation, led the statistical trends analysis. Y.H.K. contributed to the study design, data interpretation, data analysis, model evaluation. W.S.K. oversaw all aspects of study design, model evaluation, data interpretation, and the write-up.

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Table

Table 1. Clinical characteristics and the number of SNV/Indel by pathologic diagnosis.

Diagnosis	Number of patients	Age	M:F	Ann-Arbor stage		Therapeutic status		Number of SNV/Indel		
		median (range)		I or II	III or IV	Pre-Tx. (at diagnosis)	Post-Tx. (relapsed/ refractory)	Mean	Median	Range
Mature B-cell neoplasms	**243**									
Diffuse large B-cell lymphoma, NOS	154	61 (26-86)	93:61	71	83	125	29	24.84	23	11-87
Primary diffuse large B-cell lymphoma of the CNS	17	62 (34-86)	10:7	17	0	16	1	31.53	30	17-51
Primary mediastinal large B-cell lymphoma	5	36 (25-62)	3:2	1	4	5	0	31.80	32	26-41
EBV-positive diffuse large B-cell lymphoma, NOS	6	62 (19-90)	4:2	2	4	5	1	17.00	18	5-28
High-grade B-cell lymphoma	6	50.5 (37-63)	5:1	1	5	4	2	20.83	21.5	15-27
Burkitt lymphoma	1	69	0:1	0	1	0	1	22.00	22	
Plasmablastic lymphoma	2	58 (52-64)	2:0	1	1	1	1	19.50	19.5	17-22
Follicular lymphoma	29	50 (28-79)	18:11	5	24	27	2	19.62	18	8-35
Mantle cell lymphoma	11	63 (47-80)	8:3	2	9	7	4	19.09	20	11-27
Nodal marginal zone lymphoma	3	59 (58-63)	1:2	2	1	2	1	23.33	16	12-42
Extranodal marginal zone lymphoma of MALT	4	55.5 (44-68)	2:2	3	1	3	1	19.25	20.5	13-23
Lymphoplasmacytic lymphoma	2	59.5 (53-66)	2:0	0	2	0	2	17.00	17	14-20
Chronic lymphocytic leukemia/Small lymphocytic lymphoma	3	56 (53-61)	1:2	0	3	3	0	15.33	16	10-20
Mature T- and NK-cell neoplasms	**53**									
Peripheral T-cell lymphoma, NOS	10	48 (25-71)	7:3	3	7	5	5	17.60	18	11-23
Angioimmunoblastaic T-cell lymphoma	10	66.5 (43-81)	5:5	0	10	7	3	18.70	19.5	10-24
Follicular T-cell lymphoma	2	50 (48-52)	2:0	0	2	1	1	14.00	14	13-15
Nodal peripheral T-cell lymphoma with TFH phenotype	4	68.5 (64-75)	3:1	0	4	3	1	14.25	14.5	13-15
Extranodal NK/T-cell lymphoma, nasal type	18	56 (32-79)	11:7	11	7	12	6	17.44	17	9-25
Anaplastaic large cell lymphoms, ALK-positive	5	35 (20-58)	4:1	1	4	4	1	12.20	14	6-16
Anaplastaic large cell lymphoms, ALK-neative	4	40 (29-56)	3:1	1	3	4	0	22.25	23	15-28
Precursor lymphoid neoplasms	**4**									
Lymphoblastic leukemia/lymphoma	4	44.5 (34-70)	3:1	1	3	4	0	19.00	19.5	15-22
Total	**300**	**58 (19-90)**	**187:113**	**122**	**178**	**238**	**62**	**22.68**	**21**	**5-87**

SNV, single nucleotide variant; NOS, not otherwise specified.

Figures

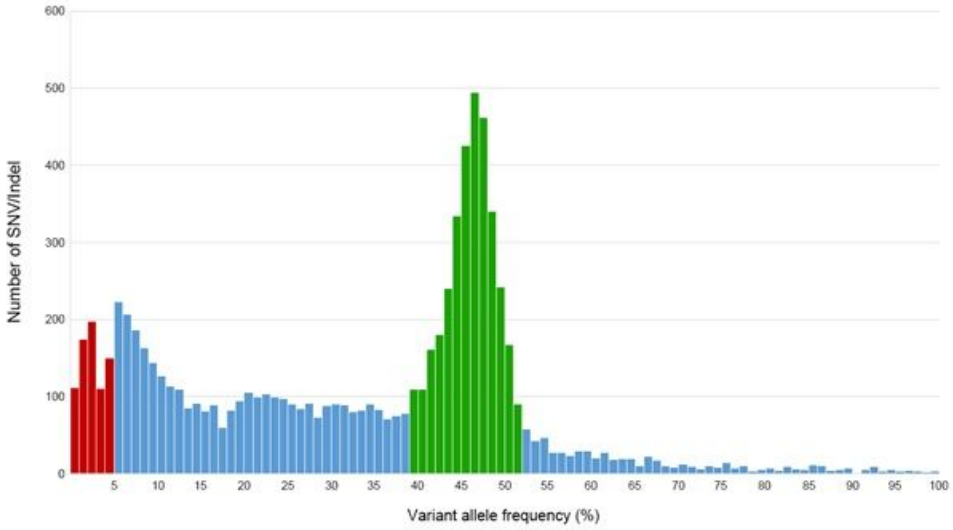

Figure 1

Number of SNV/Indel according to variant allele frequency in 300 cases. Red bars are the section with variant allele frequency less than 5%. Green bars are the section estimated to contain lots of germline mutations.

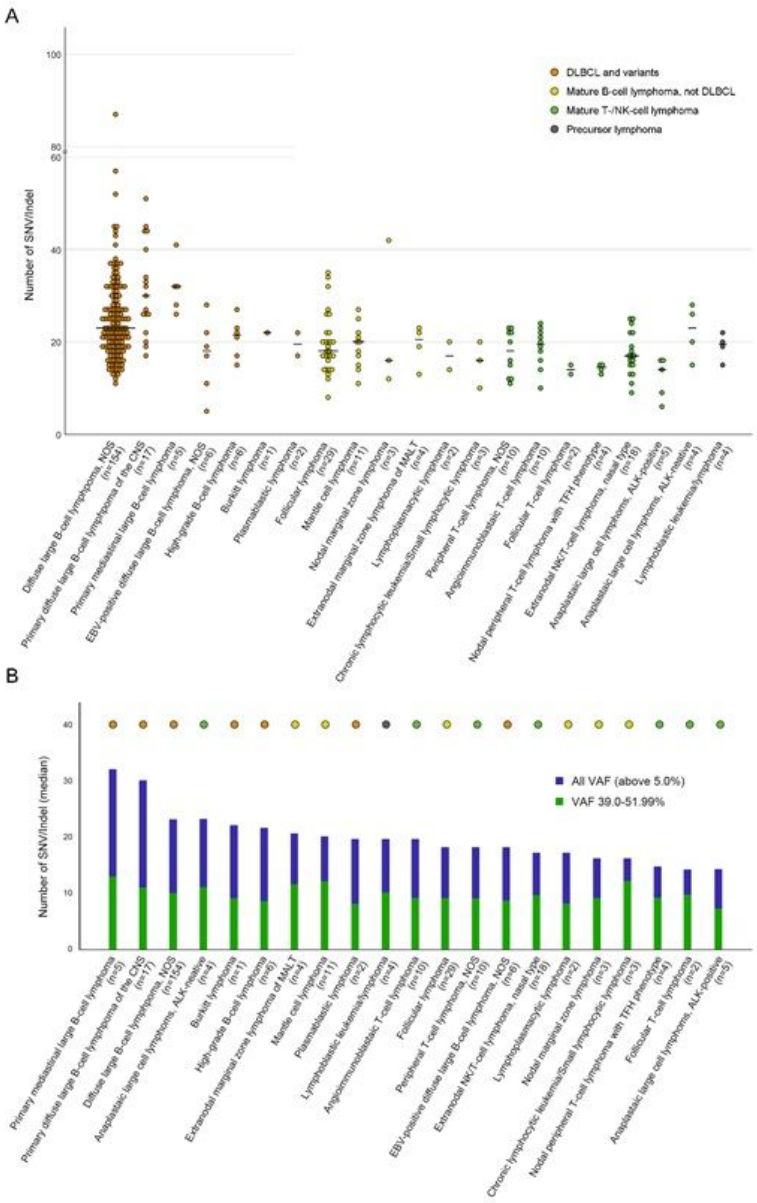

Figure 2

(A) Scatter plot of number of SNV/Indel for all cases. The horizontal bar represents the median value. (B) Bar graph in which the number of SNV/Indel is arranged in the order of median value. Blue bar represents the total number of SNV/Indel and green bar represents the number of SNV/Indel corresponding to the germline-containing VAF interval (39.0-51.99%). The classification of lymphomas is indicated by the color of dots.

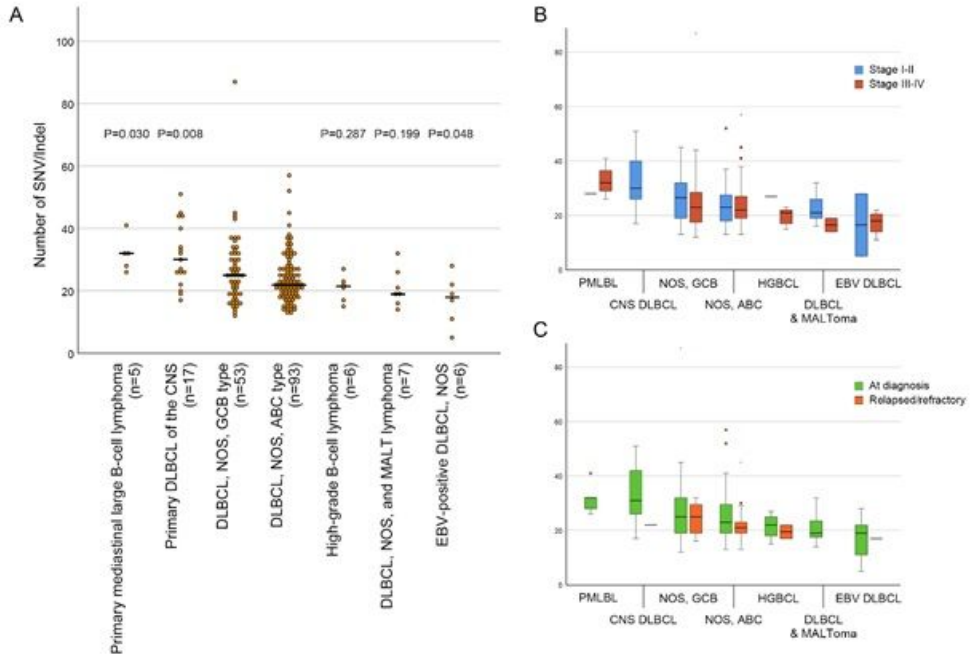

Figure 3

(A) Scatter plot showing SNV/Indel numbers of diffuse large B-cell lymphoma (DLBCL) variants. Horizontal bar represents the median value. The P value above each lymphoma is the result of Mann-Whitney U test in comparison with DLBCL, not otherwise specified (germinal center B-cell type + activated B-cell type). (B) Box plot comparing SNV/Indel numbers of DLBCL variants by Ann-Arbor stage. (C) Box plot comparing SNV/Indel numbers of DLBCL variants between tumors at diagnosis and relapsed/refractory tumors. DLBCL, diffuse large B-cell lymphoma; CNS, central nervous system; NOS, not otherwise specified, GCB, germinal center B-cell; ABC, activated B-cell; MALT, mucosa-associated lymphoid tissue, EBV, Epstein-Barr virus; PMLBL, primary mediastinal large B-cell lymphoma, HGBCL, high-grade B-cell lymphoma.

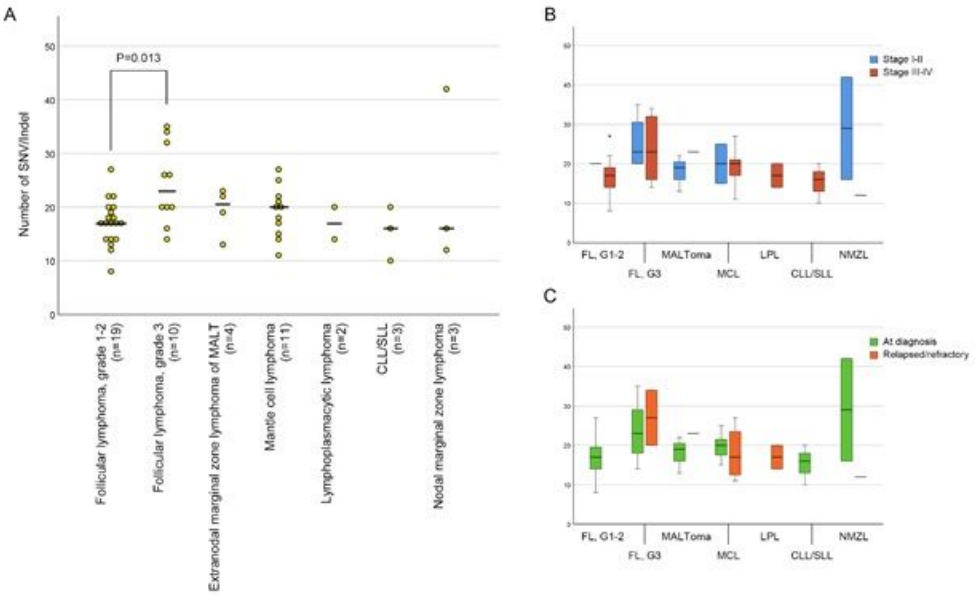

Figure 4

(A) Scatter plot showing SNV/Indel numbers of mature B-cell lymphoma except diffuse large B-cell lymphoma (DLBCL) variants. Horizontal bar represents the median value. (B) Box plot comparing SNV/Indel numbers of mature B-cell lymphoma except DLBCL variants by Ann-Arbor stage. (C) Box plot comparing SNV/Indel numbers of mature B-cell lymphoma except DLBCL variants between

tumors at diagnosis and relapsed/refractory tumors. MALT, mucosa-associated lymphoid tissue; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; MALToma, extranodal marginal zone lymphoma of MALT; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; NMZL, nodal marginal zone lymphoma.

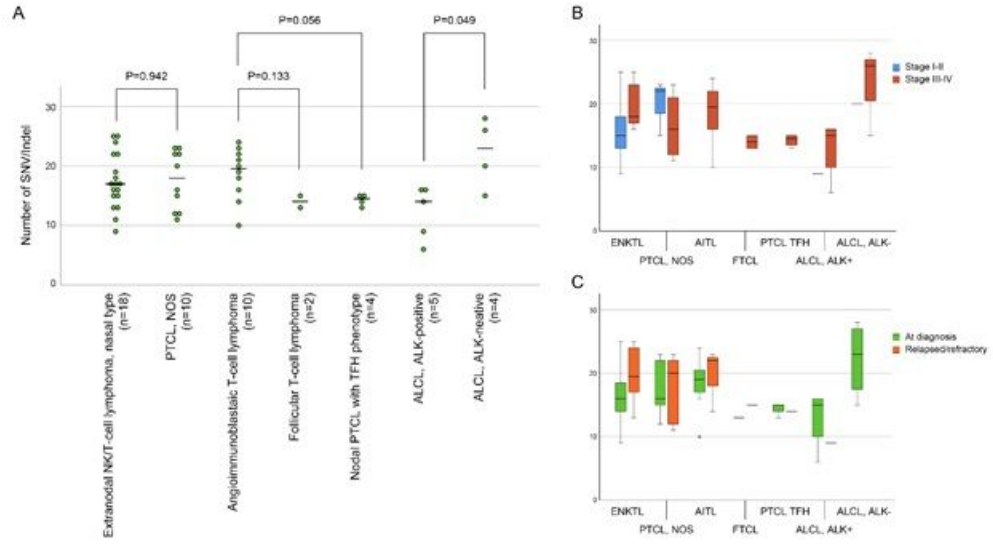

Figure 5

(A) Scatter plot showing SNV/Indel numbers of mature T- and NK- cell lymphomas. Horizontal bar represents the median value. (B) Box plot comparing SNV/Indel numbers of mature T- and NK- cell lymphomas by Ann-Arbor stage. (C) Box plot comparing SNV/Indel numbers of mature T- and NK- cell lymphomas between tumors at diagnosis and relapsed/refractory tumors. PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; TFH, T follicular helper cell; ALCL, anaplastic large cell lymphoma, ALK, anaplastic lymphoma kinase; ENKTL, extranodal NK/T-cell lymphoma, nasal type; AITL, angioimmunoblastic T-cell lymphoma; FTCL, follicular T-cell lymphoma.

Supplementary Files

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