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Research Article

Detection of Second Primary Lymphoma in Late Diffuse Large B-cell Lymphoma Recurrences

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ABSTRACT

Approximately one-third of patients with diffuse large B-cell lymphoma (DLBCL) relapse and often require salvage chemotherapy followed by autologous stem cell transplantation. In most cases, the clonal relationship between the first diagnosis and subsequent relapse is not assessed, thereby potentially missing the identification of second primary lymphoma. In this study, the clonal relationship of 59 paired DLBCL diagnoses and recurrences was established by next-generation sequencing–based detection of immunoglobulin gene rearrangements. Among 50 patients with interpretable results, 43 patients (86%) developed clonally related relapsed disease. This was observed in 100% of early recurrences (<2 years), 80% of the recurrences with an interval between 2 and 5 years, and 73% of late recurrences (≥5 years). On the other hand, 7 (14%) out of 50 patients displayed different dominant clonotypes in primary DLBCL and clinical recurrences, confirming the occurrence of second primary DLBCL; 37% of DLBCL recurrences that occurred ≥4 years after diagnosis were shown to be second primary lymphomas. The clonally unrelated cases were Epstein–Barr virus positive in 43% of the cases, whereas this was only 5% in the relapsed DLBCL cases. In conclusion, next-generation sequencing–based clonality testing in late recurrences should be considered in routine diagnostics to distinguish relapse from second primary lymphoma, as this latter group of patients with DLBCL may benefit from less-intensive treatment strategies.

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Introduction

Diffuse large B-cell lymphoma (DLBCL) displays large heterogeneity with respect to the molecular alterations that drive lymphoma development and treatment response.[1,2] Firstline

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rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone treatment achieves complete remission in approximately 60% of patients diagnosed with DLBCL. In contrast, approximately 10% suffer from primary refractory disease, with progression during or right after treatment, and 30% to 40% relapse after achieving complete remission, which is associated with poor outcomes.[3-5] DLBCL relapse predominantly occurs within 2 years after firstline immunochemotherapy,[5,6] and most of these patients will receive salvage chemotherapy regimens in combination with autologous stem cell transplantation, when eligible.[7] In approximately 15% of patients with DLBCL relapsed disease occurs after ≥5 years, and these late relapsed patients with DLBCL have a lower international prognostic index score and stage of disease, accompanied by an improved treatment outcome as compared with patients with a shorter time to relapse.[6,8,9]

B-cell lymphomas display a unique molecular footprint of immunoglobulin (IG) gene rearrangements, which facilitates the assessment of clonal relationships between diagnosis and recurrent lymphomas.[10] In case of DLBCL recurrence, B-cell clonality assessment of primary and subsequent DLBCL are generally not performed, thereby missing identification of clonally unrelated second primary DLBCL. These clinical recurrences represent de novo DLBCL, and may therefore not require intensified treatment regimens, as is the case for actual relapsed DLBCL. The underlying mechanisms for the occurrence of second primary lymphomas are still poorly understood, but it has been reported in primary immunodeficiencies and hereditary cancer predisposition syndromes.[11-13] Clonally unrelated DLBCL have only been described in relatively small cohort studies (n = 5-20 cases) in the range of 7% to 23%,[14-20] not allowing conclusions on the exact prevalence of second primary DLBCL in the general population. Therefore, it is important to obtain more information on the occurrence of second de novo DLBCL and associated clinicopathologic parameters, which will improve the diagnostics of patients with DLBCL and allow better treatment decisions.

Recently, the EuroClonality-NGS Working Group developed a novel next-generation sequencing (NGS)-based assay combined with ARResT/Interrogate immunoprofiler platform for detecting both complete and incomplete IG heavy (IGH) and IG kappa light (IGK) chain gene rearrangements.[21,22] This IG-NGS approach allows accurate clonality assessment because of the availability of the exact clonotype nucleotide sequence and is highly suitable for formalin-fixed paraffin-embedded (FFPE) samples because of the amplification of smaller-sized amplicons.[21] The IG-NGS assay has been validated for both B-cell non-Hodgkin lymphoma and classical Hodgkin lymphoma, showing improved clonality detection as compared with conventional Euroclonality-BIOMED-2/Genescan analysis.[23,24] In addition, NGS-based analysis of IG gene rearrangements allows in-depth clonotype analysis, which may facilitate the interpretation of clonality at the individual nucleotide level.

Here, we investigated the clonal relationship of DLBCL recurrences by performing IG-NGS analysis in a large cohort of paired diagnosis and recurrent DLBCL samples (n = 59), which involved different time intervals between diagnosis and recurrence(s). By determining the specific clonotypes in both the primary lymphoma and relapse, we demonstrated the occurrence of clonally unrelated lymphoma in 14% of the cases within the total cohort, all with a time to recurrence of ≥4 years and associated with Epstein–Barr virus (EBV) positivity in 43% of the clonally unrelated cases.

Materials and Methods

Patient Selection

A cohort of 59 patients was selected, diagnosed between 1991 and 2021 with de novo DLBCL (without the distinction according to the 2017 World Health Organization classification for DLBCL subtypes), followed by a subsequent DLBCL relapse for which paired tissue biopsies were available. The recurrences of patients without detailed treatment response information were considered as relapse/refractory, with an interval of <1 year following primary diagnosis (n = 6), and as relapse with ≥1 year interval. For uniformity, all cases are described as relapse in our study descriptions. Patient data and tissue samples were retrieved from the following centers: Radboud University Medical Center (Radboudumc), Rijnstate Hospital, Jeroen Bosch Hospital, Slingeland Hospital, Bernhoven Hospital, Leiden University Medical Center, and Canisius Wilhelmina Hospital. Histology of all DLBCL tissue biopsies (n = 142 in total; n = 107 FFPE and n = 35 fresh frozen [FF]), was reviewed by experienced hematopathologists (M.v.d.B. and H.v.K.). This study was approved by the local institutional Medical Ethical Committee (2020-6390).

Next-Generation Sequencing–Based Detection of Immunoglobulin Gene Rearrangements

Clonality analysis was performed by detecting (in)complete IGH chain and IGK chain gene rearrangements with NGS.[21,25] This includes IGK-deleting element (KDE) rearrangements, which in theory will be rearranged in all lambda-expressing lymphomas, whereas all kappa-expressing lymphomas will show productive IGKV-IGKJ rearrangements. For each tissue sample, 3 multiplex PCR reactions were performed with standardized primers and 40 ng input DNA (or 10 ng for limited material samples), to detect IG gene rearrangements involving variable (V), diversity (D), and joining (J) genes, as well as KDE and intronic recombination signal sequence (RSS) element. The following 5 targets were analyzed: Framework-3 (FR3) IGHV-IGHD-IGHJ, IGHD-IGHJ, IGKV-IGKJ, IGKV-KDE, and IGK-Intron RSS-KDE.

IG-NGS clonality analysis was performed using a protocol compatible with the adaptor ligation protocol with the Ion Torrent platform,[21] or a 2-step PCR protocol for sequencing on Illumina platform.[25] For the adaptor ligation protocol, library preparation was performed using the Plus Fragment Library Kit (Thermo Fisher Scientific). Thereafter, for each sample, equal DNA concentrations were loaded on Ion Torrent 318 chip (12 ng/mL) and sequenced (Ion PGM template OT2 200 kit, Ion Chef, Ion One Touch 2 system; ThermoFisher Scientific). For Illumina PCR 2-step protocol, after the first-step multiplex PCR using M13-tailed IG target specific primers,[26] library preparation was performed with adaptor-tailed M13 primers and the FastStart High Fidelity PCR System, dNTPack kit (Roche). Subsequently, the samples were pooled at equal molarity (4 nM), loaded with an Illumina mid-output chip, and sequenced with MiniSeq500. NGS data were analyzed using the bioinformatics pipeline ARResT/interrogate,[22] with a required minimum of 1000 reads coverage for IGHV-IGHD-IGHJ (FR3) and IGKV-IGKJ targets, and 500 reads for IGHD-IGHJ, IGKV-KDE, and Intron RSS-KDE targets in case of Ion Torrent protocol, and a minimum coverage of >1000 reads for all targets with the 2-step PCR Illumina protocol. A clonotype (eg, V4-34 -3/16/-1 J2) was defined by the 5′ (V4-34) and 3′ (J2) gene

annotation, and the number of nucleotides deleted from the 5′ (-3) and 3'(-1) gene and added at the junction (/16/) (including D-segment in case of IGHV-IGHD-IGHJ).[27]

Identification of Clonal Rearrangements in Diffuse Large B-cell Lymphoma Samples

To determine the lymphoma-associated clonotypes for each IG target, the abundance of the most dominant clonotype(s) was compared with the polyclonal background of nonmalignant B-cell clonotypes. The technical and molecular scoring was performed as previously described[23] (see Supplementary Materials and Methods). For the technical scoring of each target, data output was classified as not evaluable (n.e.) in case of a lower number of reads than the minimum required reads (see above), or because of lack of reproducibility in technical replicates. Data output was classified as polyclonal in case of sufficient reads but no dominant clonotype with a polyclonal background pattern, and as no clonal product (n.c.p.) with sufficient reads but low heterogeneity in polyclonal background or an irregular polyclonal pattern. Samples with interpretable results without any clonal rearrangements or only a single target, as well as all clonally unrelated cases, were repeated as a technical replicate. Recurrence-associated dominant clonotypes were traced back in the primary diagnosis of clonally unrelated cases.

Additional information regarding Materials and Methods is provided in the Supplementary Information.

Results

Clinicopathologic Characteristics of Paired Diagnosis-Recurrence Diffuse Large B-cell Lymphoma Patient Cohort

In this study, paired diagnosis—recurrence DLBCL tumor samples of 59 patients were collected for clonality assessment. The majority of patients presented with 1 DLBCL recurrence (n = 44/59, 75%), whereas 20% displayed 2 recurrences and 5% showed 3 DLBCL recurrences. (Table and Supplementary Table S1). Approximately one-third of patients presented with either low (n = 11/33; 33%) or low-to-intermediate (n = 12/33; 36%) risk international prognostic index score, and stages II, III, and IV were observed in 28% of patients each (n = 13/47). All patients were treated with (immuno-)chemotherapy (n = 47/47; 100%) after the initial diagnosis, and the majority received (immuno-)chemotherapy with or without autologous stem cell transplantation (n = 39/44, 89%) after the first recurrence (Supplementary Table S2).

The time interval between primary diagnosis and first recurrence (time to relapse) varied between 5 months and 20.3 years (median, 2.8 years), where 41% of the patients with DLBCL showed an early recurrence (<2 years), and 31% presented with a late recurrence (≥5 years). The remaining 17 patients (29%) displayed an intermediate interval between 2 and 5 years until the first relapse. In case of 2 or 3 recurrences (n = 12, 2 recurrences; n = 3, 3 recurrences), time from the first to the second recurrence involved a median interval of 2.7 years. Location of the tumor biopsies at the time of the primary diagnosis predominantly involved nodal sites (63%), with the remainder of cases presenting at extranodal locations (37%), including the skin (27%), and immune-privileged sites (23%), such as the testis and central nervous system. Tumor biopsies at recurrence were either from nodal (n = 43/77, 56%) or extranodal sites (n = 34/77, 44%).

Table
Clinicopathologic data of paired diagnosis and recurrence of DLBCL cohort

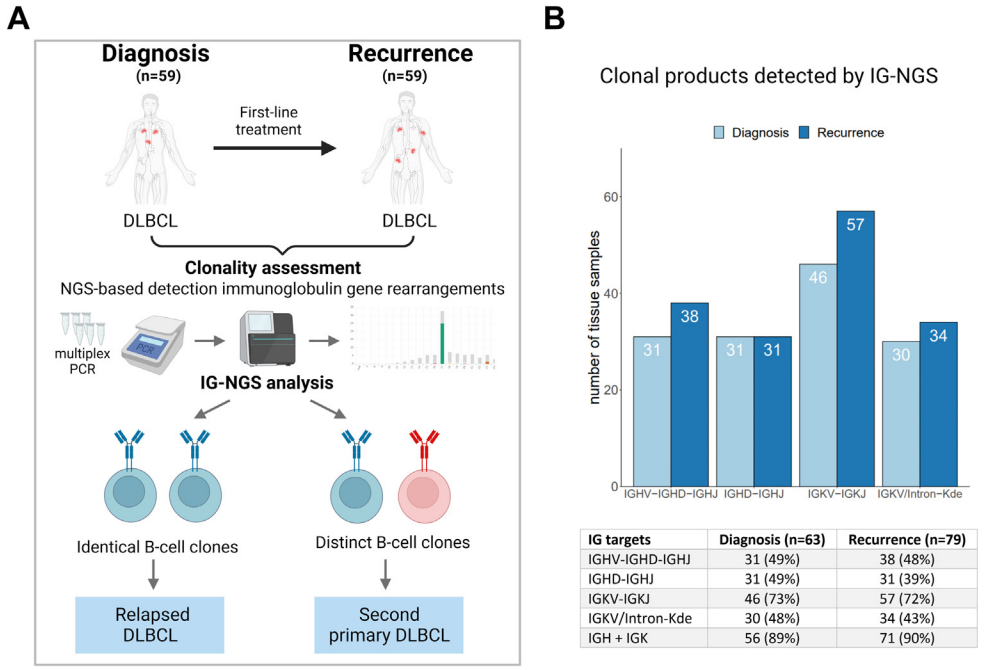
Clinicopathologic characteristics	n (%)
Gender, n = 59	
Female	26 (44%)
Male	33 (56%)
Age (y), n = 59	
<60	31 (53%)
≥60	28 (47%)
Stage, n = 47	
I	8 (17%)
II	13 (28%)
III	13 (28%)
IV	13 (28%)
IPI, n = 33	
Low	11 (33%)
Low-to-intermediate	12 (36%)
High-to-intermediate	8 (24%)
High	2 (6%)
Firstline treatment, n = 47	
Immunochemotherapy	23 (49%)
Chemotherapy	24 (51%)
Site of biopsy diagnosis, n = 59	
Nodal	37 (63%)
Extranodal	22 (37%)
Immune-privileged	5 (23%)
Number of recurrences	
1	44 (75%)
2	12 (20%)
3	3 (5%)
Time to recurrence	
Interval	0.3-20.3 y
Cell-of-origin, n = 54	
GCB	27 (50%)
Non-GCB	27 (50%)

Cell-of-origin was determined with the Hans algorithm.
In the case of nodal and extranodal biopsies, only nodal is indicated here. Immuno indicates rituximab treatment. (Immuno-)chemotherapy was combined with radiotherapy in a proportion of patients.
GCB, germinal center B-cell-like subtype; IPI, international prognostic index.

Cell-of-origin classification according to the Hans algorithm revealed an equal distribution of germinal center B-cell-like (GCB) and non-GCB subtypes as could be established for 54 cases in this study cohort (Table and Supplementary Table S1). Late recurrences (≥5 years) were predominant of GCB origin (12/18; 67%), whereas the GCB subtype was less prevalent in recurrences of <5 years (15/41; 37%). MYC- and BCL2-positive double expressor lymphomas were identified in 9 (20%) out of 46 cases, and 6 (12%) out of 50 cases showed EBV positivity, which mostly corresponded to the non-GCB subtype (Supplementary Table S2). Detection of genomic rearrangements by fluorescence in situ hybridization revealed the presence of *BCL2* translocations in 5 (15%) out of 34 cases, *BCL6* translocations in 5 (15%) out of 33 cases, and *MYC* translocations in 6 (16%) out of 37 cases. *MYC* and *BCL2* translocation-positive double-hit lymphoma was evident in 2 (5%) out of 39 cases.

Next-Generation Sequencing–Based Clonality Assessment in Diffuse Large B-cell Lymphoma Study Cohort Samples

To determine the clonal relationship in our study cohort of 59 paired diagnosis-recurrence DLBCL samples, clonality analysis was performed in a total of 142 tissue specimens (n = 107 FFPE; n = 35 FF), with the EuroClonality IG-NGS assay[21] (Fig. 1A). In each sample, the dominant clonotype(s) for every target was



IG targets	Diagnosis (n=63)	Recurrence (n=79)
IGHV-IGHD-IGHJ	31 (49%)	38 (48%)
IGHD-IGHJ	31 (49%)	31 (39%)
IGKV-IGKJ	46 (73%)	57 (72%)
IGKV/Intron-Kde	30 (48%)	34 (43%)
IGH + IGK	56 (89%)	71 (90%)

Figure 1.
Next-generation sequencing (NGS)-based clonality assessment in paired diagnosis-recurrence diffuse large B-cell lymphoma (DLBCL) cohort.
NGS-based clonality analysis in paired diagnosis-recurrence cohort of 59 patients with DLBCL. (A) Outline of the study in which immunoglobulin gene (IG)-NGS clonality data of 142 tissue specimens (formalin-fixed paraffin-embedded samples: n = 107, fresh frozen samples: n = 35) were obtained, which included 63 diagnoses (n = 4 tissue samples from 2 independent locations) and 79 recurrence samples (n = 2 tissue samples at the same time at 2 independent locations). Recurrences with identical DLBCL-associated IG gene rearrangements between diagnosis and recurrence were indicated as relapsed DLBCL, and with distinct dominant gene rearrangements as second primary lymphoma. (B) The number of tissue samples in which a dominant clonotype was identified for IG targets IGHV-IGHD-IGHJ, IGHD-IGHJ, IGKV-IGKJ, and IGKV/Intron-KDE. The results of IGHV-IGHD-IGHJ indicate FR3 target analysis.

determined according to defined guidelines (see Supplementary Information). For 6 patients, lymphoma biopsies from 2 distinct anatomical locations were included, which yielded 63 diagnosis samples and 79 recurrence samples. In 89% of the samples (n = 127/142), a dominant clonotype was detected in at least 1 of the 5 targets (Supplementary Table S3). This involved most often a clonal IGKV-IGKJ gene rearrangement (n = 103/142; 73%), which in general represents smaller-sized amplicons. Clonal IGHV-IGHD-IGHJ (FR3) gene rearrangements, which are known to be more prone to somatic hypermutations (SHMs) were detected in 49% (n = 69/142). These were in the same range as clonal nonproductive IGHD-IGHJ gene rearrangement (n = 62/142; 44%) and clonal IGKV/Intron RSS-KDE (n = 64/142; 45%) rearrangements (Fig. 1B).

IG-NGS failed to detect IG clonality in 15 out of 142 samples (11%; n = 13 FFPE; n = 2 FF), which included 7 diagnoses and 8 recurrence samples. In 9 samples this was related to inferior genomic DNA quality displaying higher fragmentation rates as confirmed by DNA integrity analysis (Supplementary Table S1), whereas in the remaining 6 samples this was most likely related to the presence of SHM in IGHV and/or IGKV regions, in combination with low tumor load (Supplementary Table S3). Four cases showed 3 or more clonal IGKV-IGKJ; IGKV/Intron-KDE rearrangements, which can be consistent with a single B-cell clone and related to the occurrence of IGK-inversions[28] (Supplementary Fig. S1).

The Clonal Relationship of Paired Diagnosis-Recurrence Diffuse Large B-cell Lymphoma

Next, the clonal relationship of the paired diagnosis-recurrence DLBCL samples was established in 50 cases for which at least 1 dominant clonotype was detected in both primary diagnosis and subsequent recurrence. In 43 (86%) of 50 cases, identical clonotypes could be detected for at least 1 of the 5 targets, indicating that diagnosis and recurrence samples were clonally related (Fig. 2; Supplementary Table S3). For 6 of the clonally related cases, differences in the junctional clonotype sequence were observed between diagnosis and relapse, most likely due to ongoing SHM (Supplementary Table S4). In contrast, 7 (14%) out of 50 cases showed different dominant clonotypes for 1 or more IG gene rearrangements and were identified as clonally unrelated recurrences. The recurrence-associated dominant clonotypes of the second unrelated DLBCL could not be detected in the primary diagnosis, except for a few reads for IGKV-KDE clonotype (but not the equivalent IGHD-IGHJ clonotype) of case DLBCL-106, which was in the range of background B cells.

In 8 cases, the clonal relationship could not be assessed because of the absence of a clonal gene rearrangement in the diagnosis sample, recurrence, or both. In addition, 1 patient (DLBCL-154), who developed 2 consecutive DLBCL recurrences (lymph node biopsies), showed clonal rearrangements with different dominant clonotypes compared with the primary DLBCL (skin biopsy) for the IGKV targets. These gene rearrangements were, however, already subclonally present in the primary DLBCL, which was also the case for the second IGHV-IGHD-IGHJ (FR3) gene rearrangement (Supplementary Table S3). Therefore, this case may represent a biclonal DLBCL at primary diagnosis, where 1 of the clones eventually relapsed. Both primary diagnosis and first recurrence were EBV positive but showed a different histomorphology, with more background inflammatory infiltrate and pleomorphic tumor cells with a Hodgkin and Reed/Sternberg cell–like morphology in the recurrence.

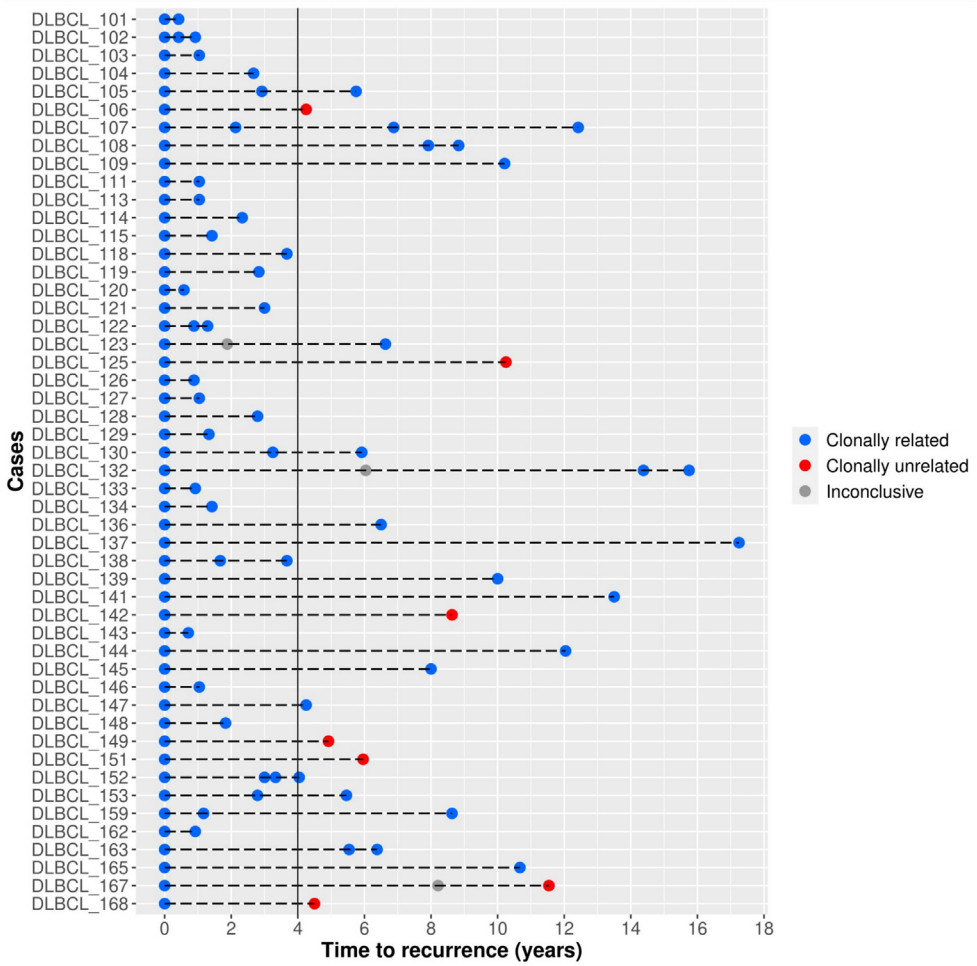


Figure 2.
Overview of clonality assessment of paired diagnosis-recurrence diffuse large B-cell lymphoma (DLBCL) cohort. Clonal relationship could be established for 50 paired diagnosis-recurrence samples, where next-generation sequencing–based clonality assessment identified DLBCL-specific clonotype(s) in both diagnosis and subsequent follow-up samples for each patient. These include clonally related DLBCL samples (n = 43; indicated in blue), clonally unrelated DLBCL samples (n = 7; indicated in red), and some intermediate samples without a detectable DLBCL-specific clonotype (n = 3), which resulted in inconclusive data (indicated in gray). Per case (indicated on the *y*-axis), the time interval between biopsies is indicated in years on the *x*-axis.

Clonally Related Diffuse Large B-cell Lymphoma Recurrences are Identified in Both Early and Late Relapsed Disease

In the 43 clonally related cases, identical clonotypes in both diagnosis and recurrence were detected for either a single IG gene rearrangement (n = 14) or 2 or more IG targets (n = 29) (Supplementary Fig. S2). Divided over different time-to-relapse intervals, 100% of early recurrences (<2 years), 80% of the recurrences with an interval between 2 and 5 years, and 73% of late recurrences (≥5 years) were clonally related (Fig. 3). Furthermore, several cases (n = 12) were patients with DLBCL with multiple recurrences in which identical clonotypes were identified. For instance, 1 case (DLBCL-107) showed 3 consecutive relapses with clonally related lymphoma and time intervals of 2.1, 4.8, and 5.5 years in between (Fig. 4). EBV positivity was observed in only 2 (5%) out of 37 clonally related DLBCL cases. Clonally related DLBCL recurrences included nodal, extranodal, and a combination of both sites for both diagnosis and relapse (Supplementary Fig. S3). Tissue biopsies taken at different anatomical locations in the same patient at the time of diagnosis (n = 6) (interval of <2 months between biopsies) were all found to be clonally related (Supplementary Tables S1 and S3).

Detection of Clonally Unrelated Diffuse Large B-cell Lymphoma in Patients With a Late Recurrence

The 7 clonally unrelated cases displayed distinct clonotypes for either 1 (n = 1), 2 (n = 4), or 3 IG targets (n = 2) (Supplementary Figs. S2 and S4). Notably, all these occurred after a 4-year interval (median 6 years, range between 4.3 and 10.3 years) (Supplementary Table S5). Within the group of DLBCL recurrences with a time to relapse of ≥4 years, 7 of 19 were clonally unrelated (37%), whereas for DLBCL recurrences with a time to relapse of ≥5 years, 4 (27%) out of 15 were clonally unrelated DLBCL. Patients who presented with second primary DLBCL had a median age of 59 years at first diagnosis (range, 43-72 years) compared with 56 years (range, 16-87 years) in relapsed DLBCL and involved no skin or immune-privileged tissue biopsies (Supplementary Table S6). Both the primary DLBCL and the clonally unrelated recurrence were the same subtype in most cases (4 cases GCB; 2 cases non-GCB). However, 1 patient presented with a GCB-DLBCL at first diagnosis, and a non-GCB at second presentation (Supplementary Table S6). Compared with the primary diagnosis of clonally related cases (n = 16/36; 44%), the GCB subtype is more prevalent in the diagnosis of clonally unrelated DLBCL samples, as it is present in 5 out of

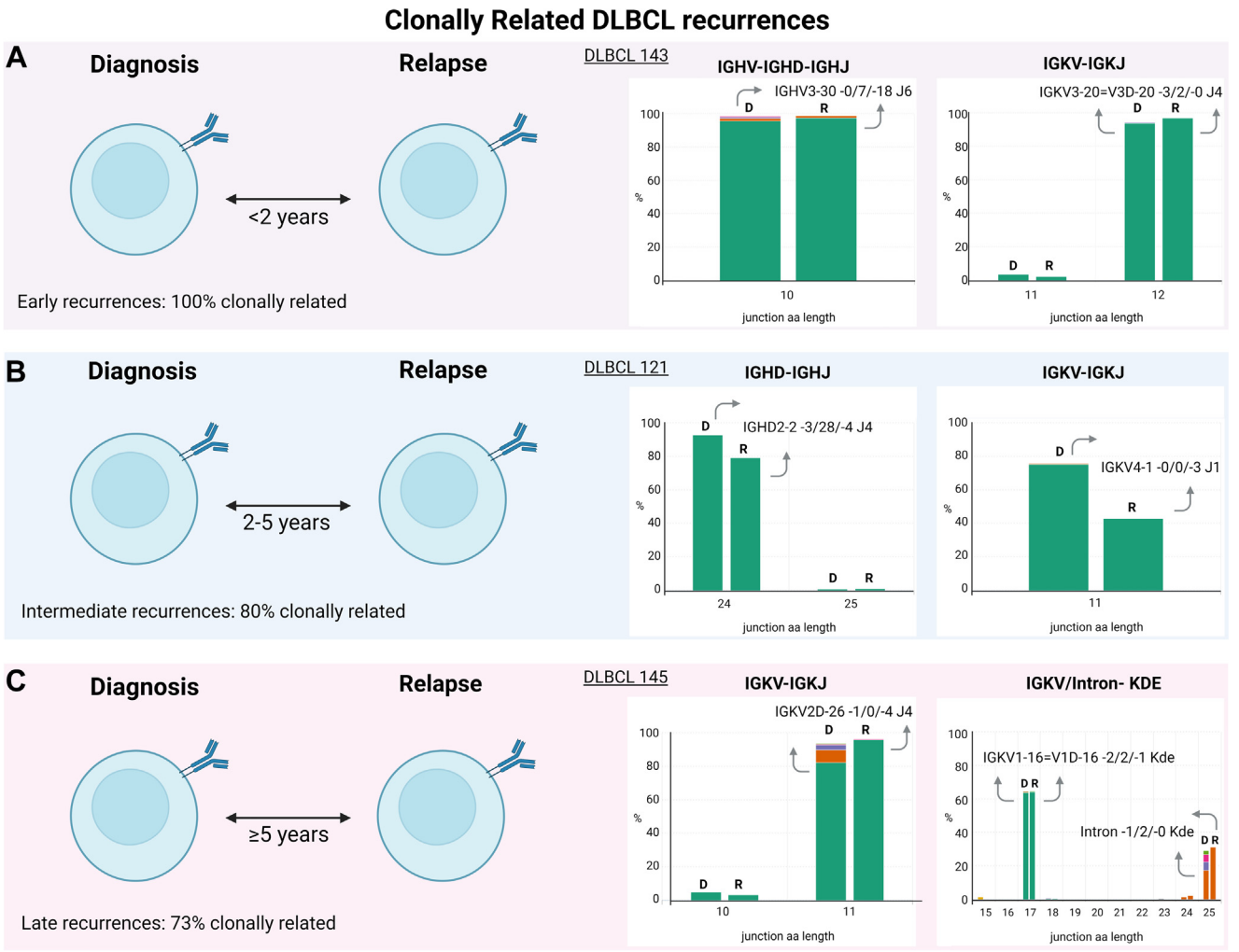


Figure 3.
Clonally related diffuse large B-cell lymphoma (DLBCL) recurrences as identified by next-generation sequencing (NGS)-based clonality assessment. Clonally related DLBCL recurrences were identified by NGS-based clonality analysis and occurred at different frequencies in early and late relapse: (A) early relapse (interval time to relapse <2 years), (B) intermediate time to relapse of 2 to 5 years, and (C) late relapse with time to relapse ≥5 years. For each time interval group, the percentage (%) of clonally related cases is indicated. A representative example for each time interval category as obtained by immunoglobulin gene (IG)-NGS analysis and ARResT/Interrogate bioinformatics is shown. The abundance of each clonotype per indicated target (IGHV-IGHD-IGHJ, IGHD-IGHJ, IGKV-IGKJ, and IGKV/Intron-KDE) is depicted on the *y*-axis and the junction amino acid (aa) length on the *x*-axis. A clonotype is defined by the 5′ and 3′ gene annotation and the junctional nucleotide sequence of the rearrangement. For each case, the dominant clonotype for diagnosis (D) and relapse (R) is indicated.

7 cases (71%). In one case (DLBCL106), the histomorphology differed between the primary and subsequent lymphoma, with an EBV-positive Hodgkin-like morphology at primary diagnosis, and an EBV-positive DLBCL morphology at second primary diagnosis (Fig. 5, Supplementary Fig. S5). EBV positivity was observed in 3 of the 7 clonally unrelated DLBCL at diagnosis (43%) (n = 2, both diagnosis and recurrence; n = 1, only at diagnosis), which is significantly higher than the EBV-positivity rate of 5% in the clonally related DLBCL cases (n = 2). The outcome data of patients with second primary DLBCL (n = 6) and relapsed DLBCL (n = 33) showed that the proportion of patients alive 4 years after recurrence was 67% for second primary DLBCL as compared with 55% in relapsed DLBCL (Supplementary Table S7).

IGHV Gene Usage in Relapsed Diffuse Large B-cell Lymphoma

To determine potential skewing in IGHV gene usage in the relapsed DLBCL cohort, the IGHV gene for each IGHV-IGHD-IGHJ gene rearrangement of the clonally related cases was analyzed (n = 35) and compared with the IGHV gene distribution in reactive lymph nodes (RLNs; n = 20) (Supplementary Table S8). IGHV4-34 represented the most abundant IGHV gene and was detected in 31% of cases as compared with 5% in RLNs (Fig. 6). Likewise, although less prevalent, IGHV3-43 was enriched in relapsed DLBCL compared with RLNs (6% vs 1%). Other IGHV genes were not different between DLBCL and RLNs, including IGHV3-23 (9% vs 8%) and IGHV4-39 (6% vs 5%), which represented other common IGHV genes in relapsed DLBCL. Because of short sequence availability of the IGHV gene within the current FR3 IG-NGS assay and high similarity among multiple IGHV3 genes, the exact IGHV3 gene usage could not be determined in 6 DLBCL cases. DLBCL cases in our relapse cohort that presented with IGHV4-34 were mostly recombined with IGHD2-21 or IGHD3-22 (each 20%) and IGHJ4 (64%) (Supplementary Table S9). Notably, 50% of IGHV4-34 cases for which we retrieved the CDR3 sequence of both diagnosis and relapse (n = 8) showed amino acid alterations (range: 1-5 amino acids) in the relapsed DLBCL junctional region, resulting

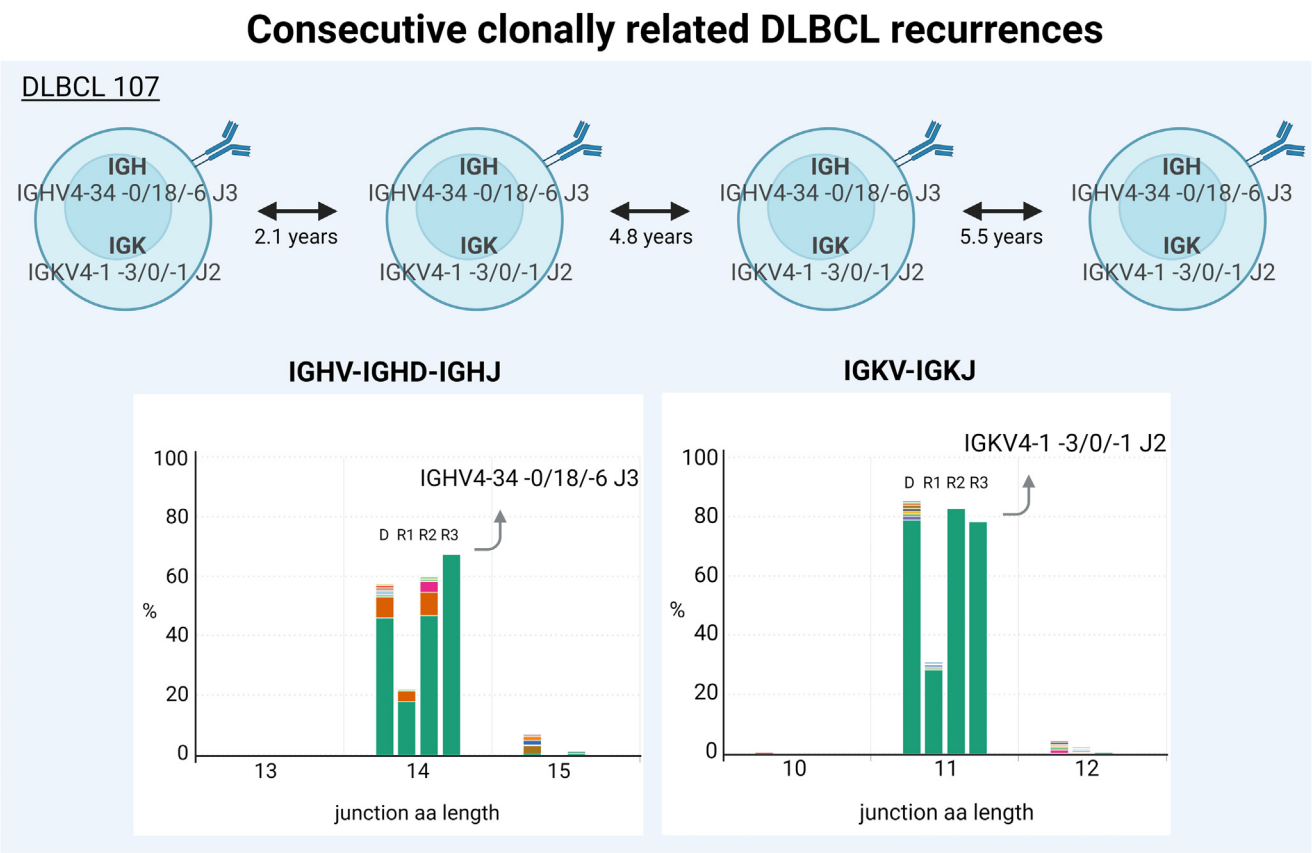


Figure 4.
Next-generation sequencing (NGS)-based clonality assessment of multiple consecutive recurrences within 1 patient. Immunoglobulin gene (IG)-NGS clonality analysis of a representative diffuse large B-cell lymphoma (DLBCL) case with multiple consecutive recurrences reveals clonally related lymphomas as determined by the presence of identical clonotypes for 2 IG gene rearrangement targets. Indicated are the time intervals in years between each relapse. Clonal gene rearrangements (indicated in green bars) of diagnosis (D), first relapse (R1), second relapse (R2), and third relapse (R3) are depicted for both IGHV-IGHD-IGHJ and IGKV-IGKJ targets. The abundance of each clonotype (each represented by a different color) is visualized on the *y*-axis and the junction amino acid (aa) length on the *x*-axis, in which a clonotype is defined by the 5′ and 3′ gene annotation and the junctional nucleotide sequence of the rearrangement.

most likely from ongoing SHM, although no common pattern or motif could be identified (Supplementary Table S9).

Discussion

Approximately 40% of patients with DLBCL suffer from refractory or relapsed disease after standard rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone treatment.[3,5] Two patterns of relapse have been proposed in DLBCL involving early-divergent/branching and late-divergent/linear evolution.[29] Disease recurrence mostly occurs within 2 years after initial diagnosis (early relapse), but in some cases, time to relapse can take >5 years (late relapse).[5,6,8,9] In both scenarios, the recurrences represent true relapsed disease or second primary DLBCL, in which case intensified treatment regimens may not be required. However, the exact prevalence of clonally unrelated DLBCL in both early and late relapse has not been firmly established.

In this study, the recently established and validated EuroClonality IG-NGS assay was employed to assess the clonal relationship of 59 paired DLBCL diagnosis and recurrence samples, including patients with multiple DLBCL recurrences. Contrary to conventional BIOMED-2/Genescan analysis, where solely fragment lengths of IG gene rearrangements can be compared (if required combined with Sanger sequencing),[20] the EuroClonality IG-NGS reveals the exact clonotype sequence, thereby providing better accuracy and overcoming potential incorrect interpretations. In this study, clonality could be assessed in 127 out of 142 DLBCL tissue samples (89%), where a higher frequency of clonal gene rearrangements of IGKV-IGKJ (73%) was detected compared with IGHV-IGHD-IGHJ (49%), due to smaller-sized amplicons and less number of SHM sites.[23] In the remaining samples, no clonal rearrangements were identified due to either poor genomic DNA quality, low tumor load, or potential mismatches between target and primer sequences related to SHM. Consequently, in 50 (85%) of the 59 cases, the clonal relationship between the primary diagnosis and recurrences could be unequivocally established. DLBCL recurrences with a time to relapse of <2 years were all clonally related to the initial DLBCL, whereas 80% of patients with an intermediate time interval between 2 and 5 years, and 73% in patients with a late relapse (≥5 years) were clonally related. Furthermore, 6 patient biopsies taken from distinct anatomical sites within a timeframe of <2 months were all clonally related.

In 1 case (DLBCL-154), the dominant clonotypes between diagnosis and recurrence were different, but the clonal relapse-associated clonotypes could be detected subclonally at diagnosis, indicating the presence of clonal heterogeneity at the time of diagnosis. Except for this 1 case, our data provide no substantial evidence for biclonality at the time of diagnosis and in relapsed

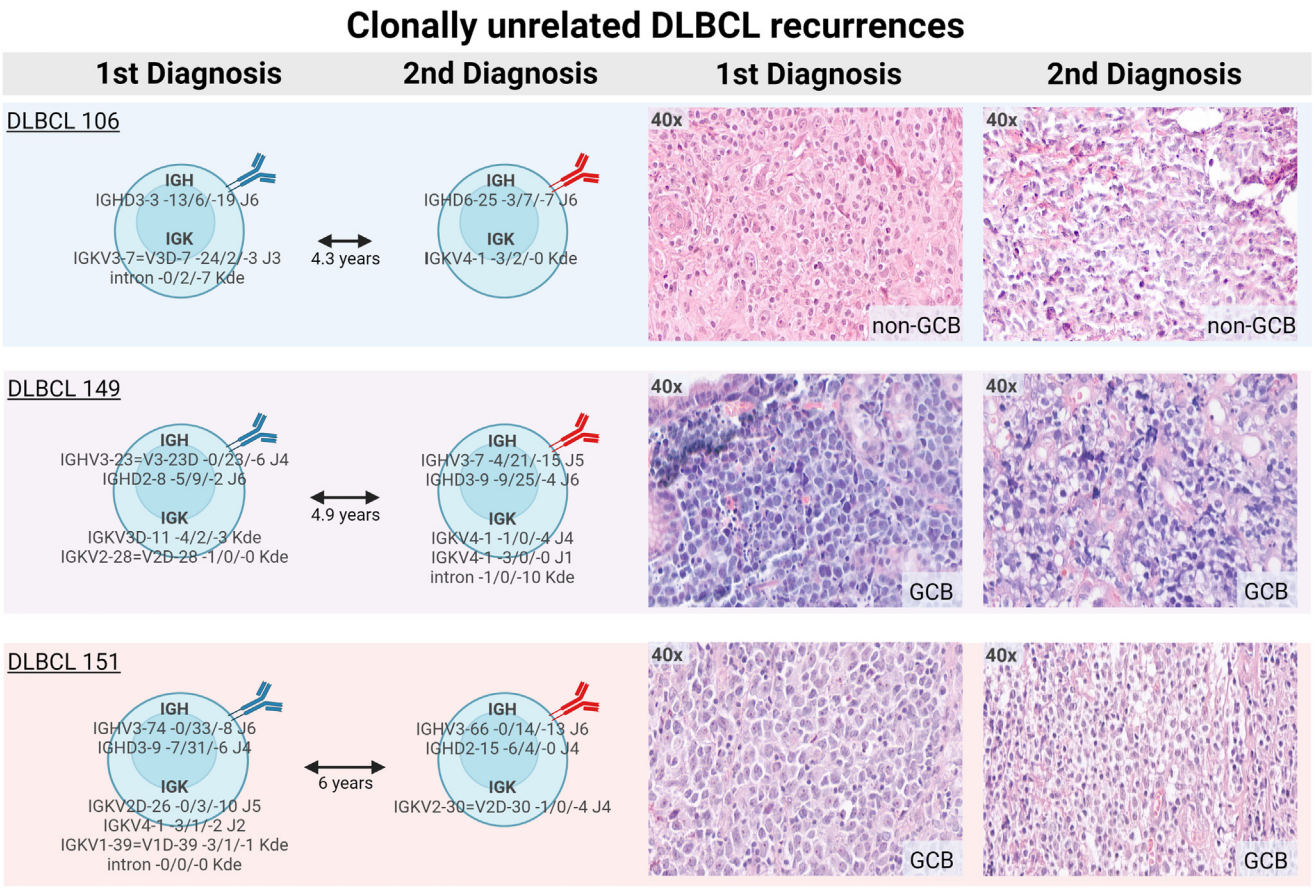


Figure 5.
Next-generation sequencing (NGS)-based clonality analysis and histopathology overview of clonally unrelated diffuse large B-cell lymphoma (DLBCL) recurrences representing second primary lymphoma. Distinct clonotypes were identified in clonally unrelated DLBCL recurrences (left panel), and the data of 3 representative cases are shown. For each clonally unrelated case, time interval in years and the dominant gene rearrangements for the specific immunoglobulin gene-targets are depicted for both first and second primary diagnoses, in which the dominant clonotype is defined by the 5′′ and 3′ gene annotation and the junctional nucleotide sequence of the rearrangement. Cell-of-origin classification (germinal center B-cell-like [GCB] and non-GCB subtypes) was determined according to the Hans algorithm. Hematoxylin and eosin (H&E) stainings (40×) for both primary and second unrelated DLBCL are shown in the right panels.

DLBCL, confirming the observation that biclonality in lymphomas is rare and mostly involves the co-occurrence of 2 distinct diseases.[30-32] Cases with multiple IGK rearrangements, possibly caused by inversion, were identified in approximately 8% (n = 4) of cases, which is in accordance with previous findings.[28]

For clonally related DLBCL recurrences, analysis of IGHV gene usage in these relapsed DLBCL cases showed a preference for IGHV4-34 compared with reactive lymphoid tissue. IGHV4-34 gene usage has been shown to correlate with chronic activated B-cell receptor signaling[33] and is required for cell survival in non−GCB-DLBCL,[34] because of autoreactivity caused by binding to glycoproteins on the cell surface.[35] The prevalence of IGHV4-34 in our cohort of relapsed DLBCL (31%) was not substantially different from primary DLBCL (approximately 24%).[33] Other frequently observed IGHV genes in relapsed DLBCL involved IGHV3-23 and IGHV4-39, but their prevalence was similar to RLNs. These common IGHV genes are known to be positively biased in comparison to other IGHV genes during V(D)J gene rearrangement in early B-cell development[36] and have also previously been identified as overrepresented in DLBCL.[37] No specific amino acid motifs could be identified within the CDR3 region. Analysis of longer IGHV gene sequences is required to provide more detailed information on clonal evolution and stereotyped B-cell receptors, particularly because binding of IGHV4-34 to self-proteins mostly relies on the FR1 region.[35]

Clonally unrelated DLBCL recurrences were observed in 14% of all cases, which is in line with previous studies in smaller DLBCL cohorts reporting unrelated lymphomas in the range of 7% to 23%.[14-17] The frequency of late recurrences (≥5 years) in our DLBCL study cohort (n = 15/50; 30%) was higher as compared with unbiased cohorts of DLBCL patients (approximately 15%).[6,8,9] This may also have affected the median age of primary diagnosis within our study cohort, which was younger (57 years) than unbiased DLBCL cohorts (65 years). However, the frequency of second primary lymphoma within the group of late recurrences should not be affected. In the clonally unrelated DLBCL samples, the dominant clonotype of the second DLBCL could not be identified as a subclone in the primary DLBCL, which excludes expansion of a minor B-cell clone already present at the time of diagnosis. All clonally unrelated second primary lymphoma in our study cohort involved patients with lymphoma with a time to recurrence of ≥4 years (Fig. 7). Previous smaller cohort studies in DLBCL have also demonstrated that clonally unrelated lymphomas mostly involve longer time-to-relapse intervals, where 70% of the patients developed an unrelated lymphoma after ≥4 years.[14-20] Currently, late DLBCL recurrences are defined by an

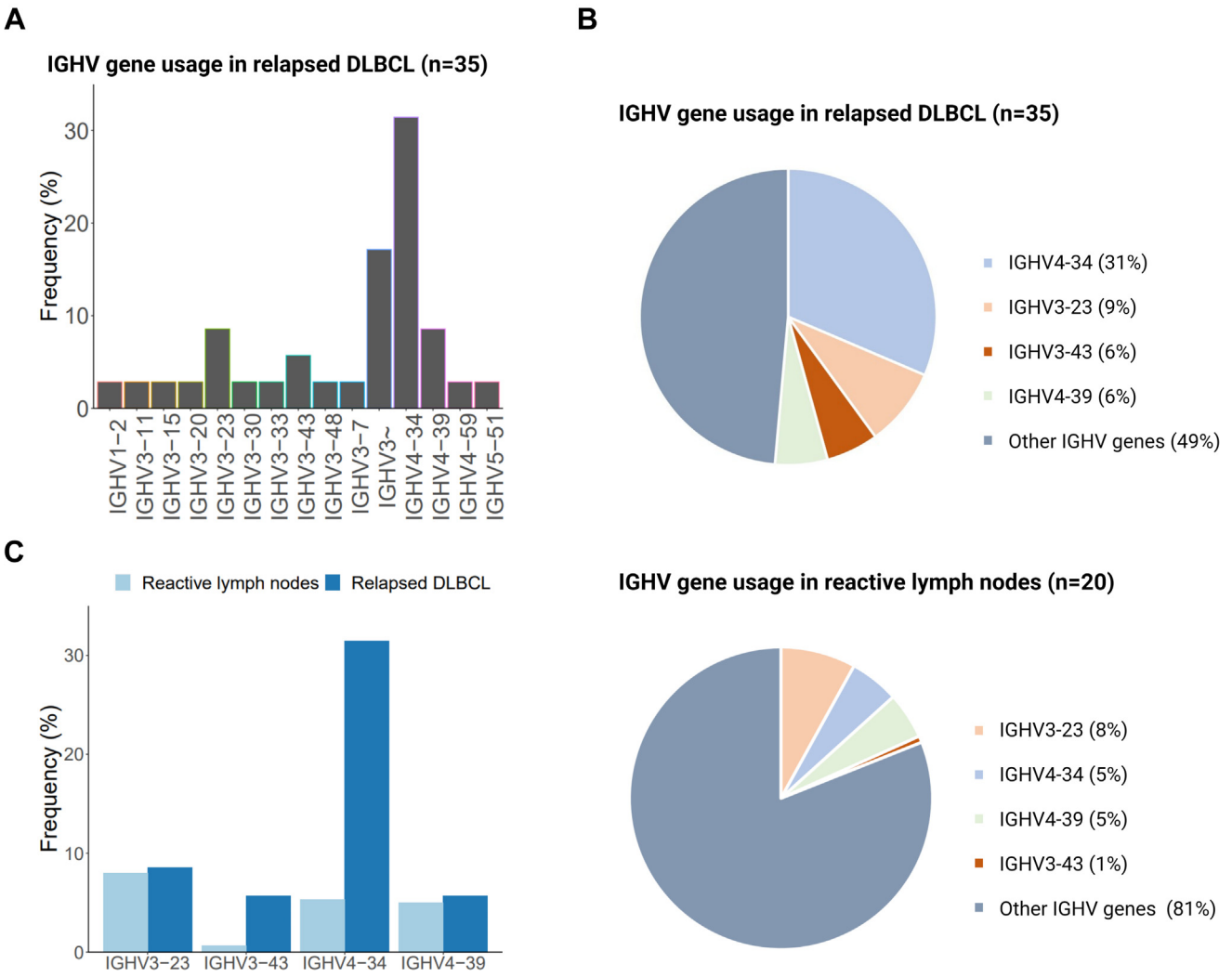


Figure 6.
IGHV gene repertoire in relapsed diffuse large B-cell lymphoma (DLBCL) cohort. Immunogenetic analysis of paired diagnosis-relapse cohort revealed IGHV gene usage in relapsed DLBCL, which was compared with IGHV gene frequencies in reactive lymph nodes. (A) Relative frequency (*y*-axis) of all IGHV genes (*x*-axis) in relapsed DLBCL cohort (n = 35). (B) Frequency of the 4 most dominant IGHV genes (present in ≥2 cases) in relapsed DLBCL (n = 35), whereas the other IGHV genes are grouped, and the relative frequency of IGHV3-23, IGHV4-34, IGHV4-39, and IGHV3-43 gene usage in reactive lymph nodes (n = 20). (C) Relative frequencies of IGHV3-23, IGHV4-34, IGHV4-39, and IGHV3-43 gene usage in relapsed DLBCL and reactive lymph nodes.

interval of ≥5 years. However, based on the accumulating data describing the occurrence of clonally unrelated DLBCL after an interval of ≥4 years, and its potential clinical impact, an interval of ≥4 years may be considered instead as a cutoff to define late recurrences. The occurrence of second primary DLBCL in the group of late recurrences, which represent actual de novo DLBCL may relate to the improved outcome within this group of patients with DLBCL as suggested by our data and as reported by others.[6,8,9]

In our study, the cell-of-origin profile according to the Hans algorithm was identical between the primary and second primary DLBCL for 6 (86%) out of 7 cases. This may suggest that susceptibility for a second de novo lymphoma within 1 patient might be linked to a specific DLBCL genetic subtype.[38,39] In addition, most of the cases showed a similar DLBCL histomorphology between primary and subsequent lymphoma, with the exception of 1 case. As could have been expected, EBV positivity was more frequent in the clonally unrelated DLBCL cases (n = 3/7; 43%) as compared with the relapse cohort (n = 2/37; 5%) (Fig. 7). Furthermore, case 154 with biclonal DLBCL was also EBV positive, indicating that EBV infection may be linked to the occurrence of multiple DLBCL clones within 1 patient. These findings suggest a role for a diminished immune response in patients with a second primary DLBCL, and/or the transforming capacity of an active EBV infection. Underlying immunodeficiency can lead to EBV reactivation and subsequent lymphoma development,[40-44] whereas second primary lymphoma may also relate to germline mutation in cancer predisposition genes.[12,45] However, no specific clinical peculiarities to suggest immune deregulation or cancer predisposition syndromes could be established in these patients.

In conclusion, second primary DLBCL were present in 37% of patients with DLBCL with a time to recurrence of ≥4 years. As such, clonality testing in cases with a longer time interval (>4 years) between the primary DLBCL and subsequent recurrence should be advised in routine diagnostics, because for patients presenting with a second unrelated lymphoma, other less-aggressive treatment options than the current intensified treatment modalities should be considered.

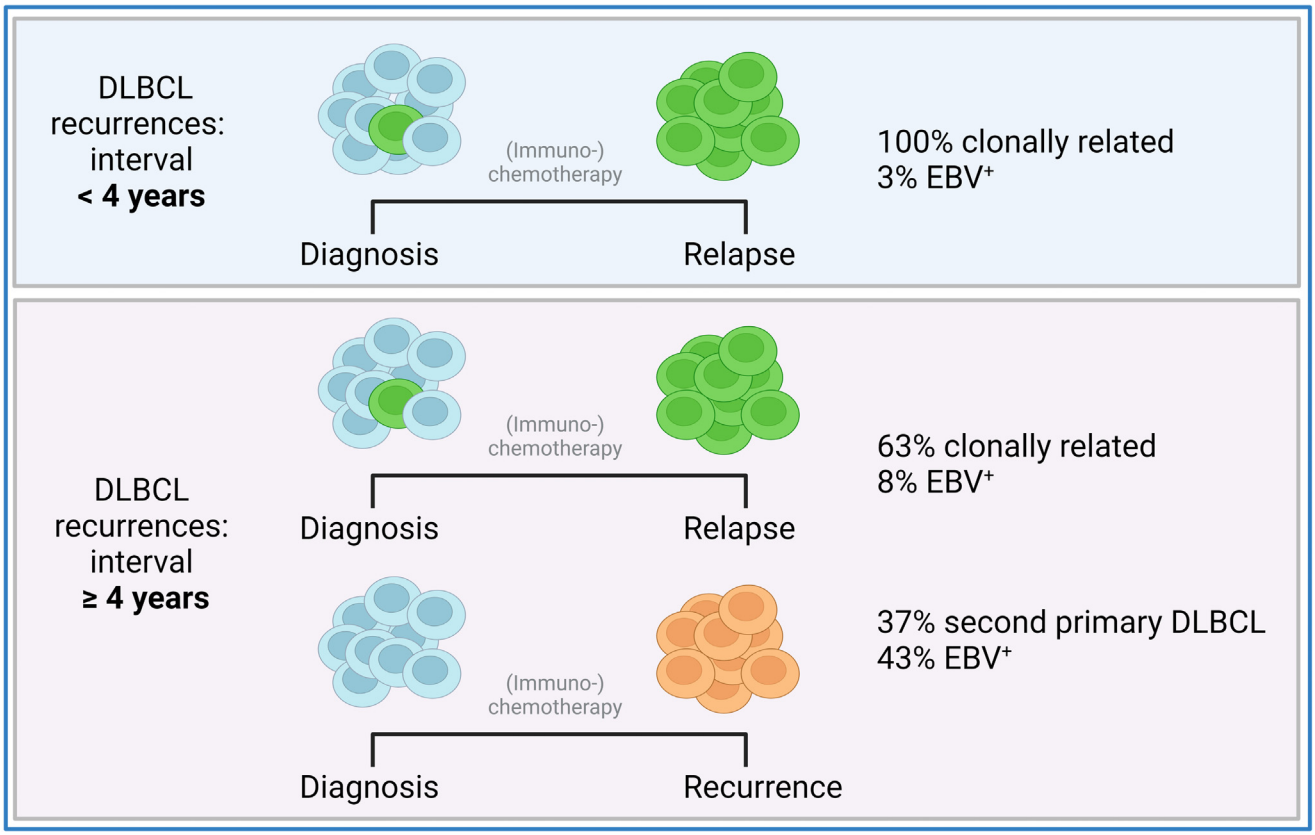


Figure 7.
Summary of second primary lymphoma in diffuse large B-cell lymphoma recurrences.

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Author Contributions

H.v.K. and B.S. designed the research and conceived the project. M.B., J.R., and J.L. performed the experimental research. M.B., D.v.B., L.K., K.H., M.v.d.B., P.G., and B.S. analyzed the data. M.B., E.H., F.d.G., B.H., A.H., P.N., E.v.B., S.d.J., C.L., H.P., E.v.d.S., J.V., C.H., W.S., and M.v.d.B. collected the histological material and/or clinical data. M.B. and B.S. wrote the manuscript. M.v.d.B. and H.v.K. examined the histopathology. All authors read and approved the final version of the paper.

Data Availability

The original raw datasets generated in this study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

The authors declare no competing financial interests.

Ethics Approval

This study received approval from the institutional CMO review board (Approval No. 2020-6390).

Supplementary Material

The online version contains supplementary material (PDF and Excel documents) available at https://doi.org/10.1016/j.modpat.2023.100119

References

1. Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. *Pathology*. 2018;50(1):74–87. https://doi.org/10.1016/j.pathol.2017.09.006
2. Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *Am J Hematol*. 2019;94(5):604–616. https://doi.org/10.1002/ajh.25460

3. Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood*. 2010;116(12):2040–2045. https://doi.org/10.1182/blood-2010-03-276246
4. Fu K, Weisenburger DD, Choi WW, et al. Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma. *J Clin Oncol*. 2008;26(28):4587–4594. https://doi.org/10.1200/JCO.2007.15.9277
5. Maurer MJ, Ghesquières H, Jais JP, et al. Event-free survival at 24 months is a robust end point for disease-related outcome in diffuse large B-cell lymphoma treated with immunochemotherapy. *J Clin Oncol*. 2014;32(10):1066–1073. https://doi.org/10.1200/JCO.2013.51.5866
6. Modvig L, Vase M, d'Amore F. Clinical and treatment-related features determining the risk of late relapse in patients with diffuse large B-cell lymphoma. *Br J Haematol*. 2017;179(1):75–82. https://doi.org/10.1111/bjh.14822
7. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med*. 1995;333(23):1540–1545. https://doi.org/10.1056/NEJM199512073332305
8. Vannata B, Conconi A, Winkler J, et al. Late relapse in patients with diffuse large B-cell lymphoma: impact of rituximab on their incidence and outcome. *Br J Haematol*. 2019;187(4):478–487. https://doi.org/10.1111/bjh.16106
9. Larouche JF, Berger F, Chassagne-Clément C, et al. Lymphoma recurrence 5 years or later following diffuse large B-cell lymphoma: clinical characteristics and outcome. *J Clin Oncol*. 2010;28(12):2094–2100. https://doi.org/10.1200/JCO.2009.24.5860
10. Libra M, De Re V, Gloghini A, Navolanic PM, Carbone A, Boiocchi M. Second primary lymphoma or recurrence: a dilemma solved by VDJ rearrangement analysis. *Leuk Lymphoma*. 2004;45(8):1539–1543. https://doi.org/10.1080/10428190310001657308
11. Obermann EC, Dirnhofer S, Tzankov A. Clonal relationship of relapsing lymphoid neoplasms. *Histol Histopathol*. 2012;27(8):1013–1020. https://doi.org/10.14670/HH-27.1013
12. Leeksma OC, de Miranda NF, Veelken H. Germline mutations predisposing to diffuse large B-cell lymphoma. *Blood Cancer J*. 2017;7(2), e532. https://doi.org/10.1038/bcj.2017.15
13. Tran H, Nourse J, Hall S, Green M, Griffiths L, Gandhi MK. Immunodeficiency-associated lymphomas. *Blood Rev*. 2008;22(5):261–281. https://doi.org/10.1016/j.blre.2008.03.009
14. Lee SE, Kang SY, Yoo HY, Kim SJ, Kim WS, Ko YH. Clonal relationships in recurrent B-cell lymphomas. *Oncotarget*. 2016;7(11):12359–12371. https://doi.org/10.18632/oncotarget.7132
15. Jiang Y, Redmond D, Nie K, et al. Deep sequencing reveals clonal evolution patterns and mutation events associated with relapse in B-cell lymphomas. *Genome Biol*. 2014;15(8):432. https://doi.org/10.1186/s13059-014-0432-0
16. de Jong D, Glas AM, Boerrigter L, et al. Very late relapse in diffuse large B-cell lymphoma represents clonally related disease and is marked by germinal center cell features. *Blood*. 2003;102(1):324–327. https://doi.org/10.1182/blood-2002-09-2822
17. Juskevicius D, Lorber T, Gsponer J, et al. Distinct genetic evolution patterns of relapsing diffuse large B-cell lymphoma revealed by genome-wide copy number aberration and targeted sequencing analysis. *Leukemia*. 2016;30(12):2385–2395. https://doi.org/10.1038/leu.2016.135
18. Lossos A, Ashhab Y, Sverdlin E, Amir G, Ben-Yehuda D, Siegal T. Late-delayed cerebral involvement in systemic non-Hodgkin lymphoma: a second primary tumor or a tardy recurrence? *Cancer*. 2004;101(8):1843–1849. https://doi.org/10.1002/cncr.20575
19. Nishiuchi R, Yoshino T, Teramoto N, et al. Clonal analysis by polymerase chain reaction of B-cell lymphoma with late relapse: a report of five cases. *Cancer*. 1996;77(4):757–762.
20. Geurts-Giele WR, Wolvers-Tettero IL, Dinjens WN, Lam KH, Langerak AW. Successive B-cell lymphomas mostly reflect recurrences rather than unrelated primary lymphomas. *Am J Clin Pathol*. 2013;140(1):114–126. https://doi.org/10.1309/AJCPI14GXNWASVUZ
21. Scheijen B, Meijers RWJ, Rijntjes J, et al. Next-generation sequencing of immunoglobulin gene rearrangements for clonality assessment: a technical feasibility study by EuroClonality-NGS. *Leukemia*. 2019;33(9):2227–2240. https://doi.org/10.1038/s41375-019-0508-7
22. Bystry V, Reigl T, Krejci A, et al. ARResT/Interrogate: an interactive immunoprofiler for IG/TR NGS data. *Bioinformatics*. 2017;33(3):435–437. https://doi.org/10.1093/bioinformatics/btw634
23. van den Brand M, Rijntjes J, Möbs M, et al. Next-generation sequencing-based clonality assessment of Ig gene rearrangements: a multicenter validation study by euroclonality-NGS. *J Mol Diagn*. 2021;23(9):1105–1115. https://doi.org/10.1016/j.jmoldx.2021.06.005
24. van Bladel DAG, van den Brand M, Rijntjes J, et al. Clonality assessment and detection of clonal diversity in classic Hodgkin lymphoma by next-generation sequencing of immunoglobulin gene rearrangements. *Mod Pathol*. 2022;35(6):757–766. https://doi.org/10.1038/s41379-021-00983-8
25. van Bladel DAG, van der Last-Kempkes JLM, Scheijen B, Groenen PJTA. Next-generation sequencing-based clonality detection of immunoglobulin gene rearrangements in B-cell lymphoma. *Methods Mol Biol*. 2022;2453:7–42. https://doi.org/10.1007/978-1-0716-2115-8_2
26. Brüggemann M, Kotrová M, Knecht H, et al. Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. *Leukemia*. 2019;33(9):2241–2253. https://doi.org/10.1038/s41375-019-0496-7
27. Knecht H, Reigl T, Kotrová M, et al. Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS. *Leukemia*. 2019;33(9):2254–2265. https://doi.org/10.1038/s41375-019-0499-4
28. Leenders AM, Kroeze LI, Rijntjes J, et al. Multiple immunoglobulin κ gene rearrangements within a single clone unraveled by next-generation sequencing-based clonality assessment. *J Mol Diagn*. 2021;23(9):1097–1104. https://doi.org/10.1016/j.jmoldx.2021.05.002
29. Juskevicius D, Dirnhofer S, Tzankov A. Genetic background and evolution of relapses in aggressive B-cell lymphomas. *Haematologica*. 2017;102(7):1139–1149. https://doi.org/10.3324/haematol.2016.151647
30. Koshy J, Dadfornia T, Qian YW. Diffuse large B-cell lymphoma arising in a composite lymphoma with biclonality by flow cytometry and one monoclonal band by PCR. *Int J Clin Exp Pathol*. 2014;7(1):407–410.
31. Fend F, Quintanilla-Martinez L, Kumar S, et al. Composite low grade B-cell lymphomas with two immunophenotypically distinct cell populations are true biclonal lymphomas. A molecular analysis using laser capture microdissection. *Am J Pathol*. 1999;154(6):1857–1866. https://doi.org/10.1016/S0002-9440(10)65443-0
32. Finch CN, Nichols M, Shrimpton A, Liu D, Hutchison RE. Primary nodal marginal zone B-cell lymphoma arising from more than one clonal neoplastic population. *Arch Pathol Lab Med*. 2000;124(12):1816–1819. https://doi.org/10.5858/2000-124-1816-PNMZBC
33. Young RM, Wu T, Schmitz R, et al. Survival of human lymphoma cells requires B-cell receptor engagement by self-antigens. *Proc Natl Acad Sci USA*. 2015;112(44):13447–13454. https://doi.org/10.1073/pnas.1514944112
34. Davis RE, Ngo VN, Lenz G, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*. 2010;463(7277):88–92. https://doi.org/10.1038/nature08638
35. Young RM, Phelan JD, Wilson WH, Staudt LM. Pathogenic B-cell receptor signaling in lymphoid malignancies: new insights to improve treatment. *Immunol Rev*. 2019;291(1):190–213. https://doi.org/10.1111/imr.12792
36. Rao SP, Riggs JM, Friedman DF, Scully MS, LeBien TW, Silberstein LE. Biased VH gene usage in early lineage human B cells: evidence for preferential Ig gene rearrangement in the absence of selection. *J Immunol*. 1999;163(5):2732–2740.
37. Sebastián E, Alcoceba M, Balanzategui A, et al. Molecular characterization of immunoglobulin gene rearrangements in diffuse large B-cell lymphoma: antigen-driven origin and IGHV4-34 as a particular subgroup of the non-GCB subtype. *Am J Pathol*. 2012;181(5):1879–1888. https://doi.org/10.1016/j.ajpath.2012.07.028
38. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403(6769):503–511. https://doi.org/10.1038/35000501
39. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346(25):1937–1947. https://doi.org/10.1056/NEJMoa012914
40. de Leval L, Vivario M, De Prijck B, et al. Distinct clonal origin in two cases of Hodgkin's lymphoma variant of Richter's syndrome associated with EBV infection. *Am J Surg Pathol*. 2004;28(5):679–686. https://doi.org/10.1097/00000478-200405000-00018
41. Mao Z, Quintanilla-Martinez L, Raffeld M, et al. IgVH mutational status and clonality analysis of Richter's transformation: diffuse large B-cell lymphoma and Hodgkain lymphoma in association with B-cell chronic lymphocytic leukemia (B-CLL) represent 2 different pathways of disease evolution. *Am J Surg Pathol*. 2007;31(10):1605–1614. https://doi.org/10.1097/PAS.0b013e31804bdaf8
42. Latour S, Winter S. Inherited immunodeficiencies with high predisposition to Epstein-Barr virus-driven lymphoproliferative diseases. *Front Immunol*. 2018;9:1103. https://doi.org/10.3389/fimmu.2018.01103
43. Iglesias Cardenas F, Agarwal AM, Vagher J, Maese L, Fluchel M, Afify Z. Two clonally distinct B-cell lymphomas reveal the diagnosis of XLP1 in a male child and his asymptomatic male relatives: case report and review of the literature. *J Pediatr Hematol Oncol*. 2021;43(8):e1210–e1213. https://doi.org/10.1097/MPH.0000000000002049
44. López-Nevado M, González-Granado LI, Ruiz-García R, et al. Primary immune regulatory disorders with an autoimmune lymphoproliferative syndrome-like phenotype: immunologic evaluation, early diagnosis and management. *Front Immunol*. 2021;12, 671755. https://doi.org/10.3389/fimmu.2021.671755
45. Gładkowska-Dura M, Dzierzanowska-Fangrat K, Dura WT, et al. Unique morphological spectrum of lymphomas in Nijmegen breakage syndrome (NBS) patients with high frequency of consecutive lymphoma formation. *J Pathol*. 2008;216(3):337–344. https://doi.org/10.1002/path.2418