A significant proportion of classic Hodgkin lymphoma recurrences represents clonally unrelated second primary lymphoma

Bladel, D.A.G. van; Stevens, W.B.C.; Kroeze, L.I.; Groen, R.A.L. de; Groot, F.A. de; Last-Kempkes, J.L.M. van der; ... ; Scheijen, B.

REGULAR ARTICLE

blood advances

A significant proportion of classic Hodgkin lymphoma recurrences represents clonally unrelated second primary lymphoma

Diede A. G. van Bladel,[1] Wendy B. C. Stevens,[2] Leonie I. Kroeze,[1] Ruben A. L. de Groen,[3] Fleur A. de Groot,[3] Jessica L. M. van der Last-Kempkes,[1] Madeleine R. Berendsen,[1] Jos Rijntjes,[1] Jeroen A. C. W. Luijks,[1] Irina Bonzheim,[4] Ellen van der Spek,[5] Wouter J. Plattel,[6] Johannes F. M. Pruijt,[7] Susan D. P. W. M. de Jonge-Peeters,[8] Gerjo A. Velders,[9] Chantal Lensen,[10] Esther R. van Bladel,[11] Birgit Federmann,[4,12] Brigiet M. Hoevenaars,[13] Agata Pastorczak,[14] Jutte van der Werff ten Bosch,[15,16] Joost S. P. Vermaat,[3] Peet T. G. A. Nooijen,[17] Konnie M. Hebeda,[1] Falko Fend,[4] Arjan Diepstra,[18] J Han J. M. van Krieken,[1] Patricia J. T. A. Groenen,[1] Michiel van den Brand,[19] and Blanca Scheijen[1]

[1]Department of Pathology and [2]Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands; [3]Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands; [4]Institute of Pathology and Neuropathology, Comprehensive Cancer Center, University Hospital Tübingen, Tübingen, Germany; [5]Department of Hematology, Rijnstate Hospital, Arnhem, The Netherlands; [6]Department of Hematology, University Medical Center Groningen, Groningen, The Netherlands; [7]Department of Hematology, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands; [8]Department of Hematology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; [9]Department of Internal Medicine, Gelderse Vallei Hospital, Ede, The Netherlands; [10]Department of Hematology, Bernhoven Hospital, Uden, The Netherlands; [11]Department of Internal Medicine, Slingeland Hospital, Doetinchem, The Netherlands; [12]Department of Translational Immunology, German Cancer Research Center, Medical Hospital Tübingen, Tübingen, Germany; [13]Department of Pathology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; [14]Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Lodz, Poland; [15]Department of Pediatric Hematology and Oncology, University Hospital Brussels, Brussels, Belgium; [16]Department of Pediatrics, Paola Children's Hospital, Antwerp, Belgium; [17]Pathology-DNA, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands; [18]Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, The Netherlands; and [19]Pathology-DNA, Rijnstate Hospital, Arnhem, The Netherlands

Key Points

- A considerable subset of classic Hodgkin lymphoma recurrences represents clonally unrelated second primary lymphoma.
- Assessment of T-cell clonality combined with pathogenic mutation analysis can identify TCL mimicking cHL.

Despite high cure rates in classic Hodgkin lymphoma (cHL), relapses are observed. Whether relapsed cHL represents second primary lymphoma or an underlying T-cell lymphoma (TCL) mimicking cHL is underinvestigated. To analyze the nature of cHL recurrences, in-depth clonality testing of immunoglobulin (Ig) and T-cell receptor (TCR) rearrangements was performed in paired cHL diagnoses and recurrences among 60 patients, supported by targeted mutation analysis of lymphoma-associated genes. Clonal Ig rearrangements were detected by next-generation sequencing (NGS) in 69 of 120 (58%) diagnoses and recurrence samples. The clonal relationship could be established in 34 cases, identifying clonally related relapsed cHL in 24 of 34 patients (71%). Clonally unrelated cHL was observed in 10 of 34 patients (29%) as determined by IG-NGS clonality assessment and confirmed by the identification of predominantly mutually exclusive gene mutations in the paired cHL samples. In recurrences of >2 years, ~60% of patients with cHL for whom the clonal relationship could be established showed a second primary cHL. Clonal TCR gene rearrangements were identified in 14 of 125 samples (11%), and TCL-associated gene mutations were detected in 7 of 14 samples. Retrospective pathology review with integration of the molecular findings were consistent with an underlying TCL in 5 patients aged >50 years. This study shows that cHL recurrences, especially after 2 years, sometimes represent a new primary cHL or TCL mimicking cHL, as uncovered by NGS-based Ig/TCR clonality testing and gene mutation analysis. Given the significant therapeutic consequences, molecular testing of a presumed relapse in cHL is crucial for subsequent appropriate treatment strategies adapted to the specific lymphoma presentation.

Submitted 7 April 2023; accepted 19 July 2023; prepublished online on *Blood Advances* First Edition 8 August 2023; final version published online 29 September 2023. https://doi.org/10.1182/bloodadvances.2023010412.

Data are available on request from the corresponding author, Blanca Scheijen (blanca.scheijen@radboudumc.nl).

The full-text version of this article contains a data supplement.

Introduction

Hodgkin lymphoma (HL) affects both young adults and older patients and has an age-adjusted incidence of ~3 in 100 000 people in the Western world.[1] HL can be divided into 2 distinct entities, nodular lymphocyte–predominant HL and classic HL (cHL), of which cHL is the most common form, representing a spectrum of 4 different morphological subtypes.[2] Current multiagent chemotherapy with or without radiotherapy results in high cure rates of patients with cHL, with a 5-year overall survival exceeding 90%.[3-5] However, up to 25% of patients with cHL show refractory or relapsed disease, often involving patients with advanced-stage disease.[6-8] In most patients, relapsed disease develops within 2 years after the primary diagnosis, but recurrences may even occur after 5 years.[9-11]

The lymphoid neoplasm cHL originates from transformed germinal center B cells. The malignant $CD30^+$ Hodgkin and Reed-Sternberg (HRS) cells have lost most phenotypic B-cell characteristics, including the expression of the B-cell receptor, but clonal immunoglobulin (IG) gene rearrangements are still detectable.[12] This molecular fingerprint has confirmed the clonal outgrowth of malignant B cells and can serve as a diagnostic marker to establish the clonal relationship between primary cHL and its recurrences.[12,13] Because of the limited number of HRS cells (usually <1%-5%) in an inflammatory background, Ig clonality assessment in whole tissue specimens has been challenging in a diagnostic setting. The recently developed EuroClonality IG–next-generation sequencing (NGS) assay,[14,15] which has shown high sensitivity, allows for improved detection of malignant HRS clones in cHL tissues.[16] Besides detection of Ig clonality, clonal T-cell receptor (TR) gene rearrangements have been observed in cHL,[17,18] even in isolated $CD30^+$ HRS cells,[19-23] suggesting a T-cell origin in very rare cases of cHL. More common are T-cell lymphomas (TCLs), with atypical B cells mimicking cHL, such as nodal T-follicular helper cell lymphomas (nTFHLs), including both the angioimmunoblastic[24-26] and the follicular types.[25,27,28]

In cases in which it remains challenging to distinguish cHL from TCL with HRS-like cells, gene mutation analysis on tissue samples can be helpful to complement clinicopathological evaluation. Recurrently mutated genes driving cHL pathogenesis include *SOCS1*, *TNFAIP3*, and *B2M*,[29-31] whereas genetic alterations associated with the angioimmunoblastic type of nTFHL, here referred to as angioimmunoblastic TCL (AITL), commonly involve *RHOA*, *TET2*, *DNMT3A*, and *IDH2*.[32,33] Notably, some of these affected genes, such as *TET2* and *DNMT3A* mutations, are drivers of clonal hematopoiesis of indeterminate potential (CHIP), which predisposes to hematologic malignancies,[34,35] and can also be detected in HRS cells themselves.[36]

It is commonly assumed that recurrence of cHL after treatment is a relapse of the original disease. However, we hypothesize, in line with a previous study,[37] that some patients may actually present with a new primary cHL, unrelated to the original tumor. Therefore, we analyzed paired diagnoses and recurrence tissue samples in a cohort of 60 patients with cHL to determine the clonal relationship between these cHL presentations by performing NGS-based Ig and TCR clonality assessment and targeted mutation analysis. Our in-depth molecular analysis demonstrated that recurrences of cHL represent clonally unrelated second primary cHL in a considerable proportion of cases. Moreover, several cHL and its recurrences appeared upon retrospective pathology review TCL with HRS-like cells mimicking cHL.

Methods

Patient cohort and tissue samples

The study cohort consisted of 60 patients whose tissue samples were available from both cHL primary diagnosis and secondary disease. Archival material from 1995 to 2019 was obtained from pathology departments within the Netherlands (Radboud University Medical Center, Canisius Wilhelmina Hospital, Rijnstate Hospital, Jeroen Bosch Hospital, and University Medical Center Groningen), Germany (University Hospital Tübingen), Poland (Medical University of Lodz), and Belgium (University Hospital Brussels). Each tissue sample was reviewed by 2 experienced hematopathologists (H.v.K. and M.v.d.B.) according to the 2017 World Health Organization classification.[2] Clinical and pathological information is summarized in supplemental Table 1. Tissue biopsy specimen from recurrences in patients with an interval of ≤1 year after primary diagnosis and without confirmed complete response (CR; n = 4; cases 20, 30, 31, and 52) were still considered as relapses in our study descriptions. An age-matched cHL control cohort of patients without a relapse (median follow-up of 16 years) was included to establish whether TCR clonality was associated with relapsed cHL (supplemental Table 2). All samples and clinical information were collected in accordance with the Declaration of Helsinki and Declaration of Taipei and received approval of the local medical ethical review board (approval number #2020-6390).

NGS-based clonality assessment

NGS-based clonality assays were performed as previously described by the EuroClonality-NGS Working Group[14,38,39] to detect Ig heavy chain (IGH) and Ig kappa light chain (IGK) gene rearrangements or TRB and TRG gene rearrangements. The 5 standard targets of the EuroClonality Ig-NGS assay (framework 3 [FR3] IGHV-IGHD-IGHJ, IGHD-IGHJ, IGKV-IGKJ, and IGKV/intron recombination signal sequence [RSS]- kappa deleting element [KDE]) were analyzed for all samples. Samples without detectable IG gene rearrangements for these 5 targets and sufficient DNA quality were subsequently tested for FR2 IGHV-IGHD-IGHJ gene rearrangements (supplemental Materials and Methods; supplemental Tables 3 and 4). IG-NGS clonality assessment was performed in duplicates with 40- to 80ng DNA input. TR-NGS clonality assay involved detection of TRBV-TRBD-TRBJ, TRBD-TRBJ, and TRGV-TRGJ gene rearrangements and was performed with 40ng DNA input for each polymerase chain reaction. Library preparations were made compatible for sequencing either on an IonTorrent or Illumina platform. NGS data sets (Bioproject accession number PRJNA1003679) were analyzed using the bioinformatics analysis tool ARResT/Interrogate (http://arrest.tools/interrogate).[40] Guidelines for Ig clonality assessment in cHL have been described previously,[16] and for TCR clonality detection, it is provided in the supplemental Materials and Methods.

Mutation analysis. Mutation analysis of clonally unrelated cHL was performed with the Trusight Oncology 500 (TSO500; Illumina, San Diego, CA) NGS panel (supplemental Table 5A) on total

genomic DNA and sequenced on an Illumina NextSeq500 platform, as described previously.[41] Lymphomas with clonal TR gene rearrangements were analyzed with AmpliSeq Custom panels (AITL[32] and BLYMF200[42]; supplemental Table 5B-C, respectively) and sequenced on an IonTorrent platform. Further details are described in the supplemental Information.

Results

Clinicopathological characteristics of paired diagnosis and recurrence in the cHL patient cohort

The study cohort consisted of archived paired diagnosis and secondary cHL tissue samples from 60 patients diagnosed with cHL based on clinical and histomorphological criteria. A total of 130 tissue samples were included, which involved 99 formalin-fixed, paraffin-embedded (FFPE) and 31 fresh frozen (FF) tissue specimens. Within this cohort, the median age at the time of primary diagnosis was 26 years (range, 4-76 years), and 18 patients were diagnosed with cHL in childhood (age, ≤18 years; Table 1). At primary cHL diagnosis, 55% of the patients presented with advanced-stage disease (Ann Arbor stage III-IV), which is higher than that of the general cHL population (30%).[43] The majority of primary cHL tissue samples showed nodular sclerosis (NSHL) morphology (n = 34, 57%), followed by mixed cellularity (MCHL; n = 22, 37%). Epstein-Barr virus (EBV)–positive HRS cells were detected in 13 of 60 (22%) primary cHL diagnosis samples and in 17 of 68 (25%) of the recurrences (supplemental Table 6). Considering all diagnosis and recurrence samples with known EBV status (n = 114), EBV positivity was more frequently present in MCHL (39%) than in NSHL (16%; $P = .007$). The median time to the first recurrence was 1.5 years (range, 0.4-13.6 years), and 25 patients (42%) developed a first recurrence >2 years after primary diagnosis. From 12 patients, 2 independent recurrences were included for molecular analysis.

Identification of clonally related relapsed cHL by NGS-based detection of IG gene rearrangements

Clonality analysis was performed with the EuroClonality IG-NGS assay, and in 120 of 130 tissue samples, good quality NGS clonality data sets were obtained with interpretable results. Clonal IG gene rearrangements were detected in 69 of 120 samples (58%; 46 of 89 FFPE = 52%; 23 of 31 FF = 74%), whereas 51 samples (43%; 43 of 89 FFPE = 48%; 8 of 31 FF = 26%) displayed a polyclonal pattern (supplemental Table 7). In the remaining 10 samples (7 primary diagnoses and 3 recurrences), IG-NGS generated poor-quality data sets that were noninterpretable, mainly because of inferior genomic DNA quality. Therefore, cHL tissue samples of 7 of the 60 patients had to be excluded from subsequent analysis. In the other 53 patients, Ig clonotype comparison between primary diagnosis and recurrences revealed identical Ig-related clonotypes in 16 patients, confirming their clonal relationship and thus an actual relapse (supplemental Table 7). In 15 of the remaining 37 patients, a dominant Ig clonotype could be detected in only 1 of the cHL tissue samples, whereas the paired sample(s) lacked detection of a clonal Ig gene rearrangement. However, backtracking of the specific cHL-associated clonotypes in the 15 reciprocal samples resulted in the identification of identical clones below the defined clonality thresholds in 5 additional cases (see supplemental Information for clonality threshold definitions).

Table 1. Clinicopathological data of paired diagnosis and recurrence cHL cohort at the time of primary diagnosis

Total cohort (N = 60)	n (%)
Sex (N = 60)	
Male	37 (62)
Female	23 (38)
Median age (N = 60)	26 y (4-76 y)
Pediatric (≤18 y)	18 (30)
Adults (>18 y)	42 (70)
Disease stage Ann Arbor (n = 52)	
I	6 (12)
II	19 (37)
III	17 (33)
IV	10 (19)
Risk groups (n = 40)	
Stages I to II (n = 24)	
EORTC favorable	9 (23)
EORTC unfavorable	15 (38)
Stages III to IV (n = 16)	
IPS 0 to 2	8 (20)
IPS ≥3	8 (20)
First line treatment (n = 52)*	
Chemotherapy	49 (94)
Radiotherapy	20 (38)
Immunotherapy	1 (2)
Time to first recurrence (N=60)	
Median interval (range) (y)	1.5 (0.4-13.6)
cHL subtype (N = 60)	
NSHL	34 (57)
MCHL	22 (37)
LRHL	1 (2)
cHL-NOS	3 (5)
EBV status (N = 60)	
Negative	47 (78)
Positive	13 (22)

EORTC, European Organization of Research and Treatment of Cancer; IPS, international prognostic score; LRHL, lymphocyte rich Hodgkin lymphoma; NOS, not otherwise specified.
*If patients received combination therapy, multiple treatment categories were applicable, making the sum of all categories >100%. For detailed information, see supplemental Table 1.

Therefore, these 5 samples were also classified as clonally related cHL cases (supplemental Figure 1). In addition, NGS-based detection of FR2 IGHV-IGHD-IGHJ gene rearrangements was performed for a selected set of unresolved cases with sufficient DNA quality (n = 14 cases; n = 29 samples). This revealed detectable clonal IG gene rearrangements in 10 of 29 samples (supplemental Table 7), thereby identifying 3 additional clonally related cases. Collectively, NGS-based clonality assessment by detection of IG gene rearrangements demonstrated clonally related relapsed cHL in 24 patients (Figure 1).

The majority of patients with clonally related cHL developed a relapse within 2 years after their primary diagnosis (19 of 24

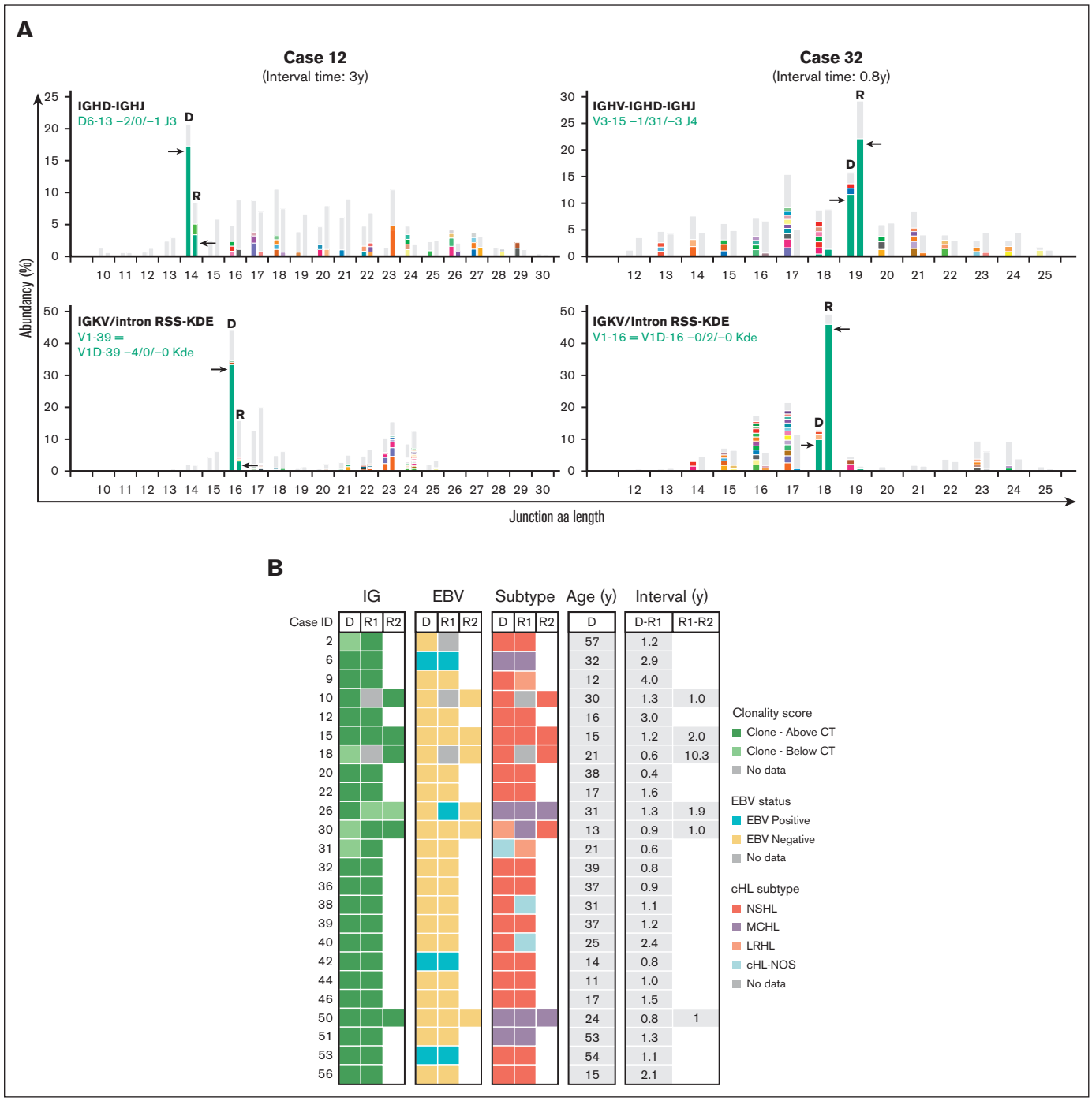

Figure 1. NGS-based clonality analysis identifies clonally related cHL recurrences. (A) Representative data sets indicating NGS-based detection of IG gene rearrangements in paired diagnosis and recurrence tissue samples of 2 patients with clonally related relapsed cHL (cases 12 and 32). For each patient, the data for 2 clonal IG gene rearrangements are shown; IGKV-IGKJ and IGKV/intron RSS-KDE for case 12 and IGHV-IGHD-IGHJ and IGKV/intron RSS-KDE for case 32. The specific clonotype for the dominant IG gene rearrangement is indicated in green. On the x-axis, the junction length in amino acids (junction aa length) is shown, and the abundancy of clonotypes is shown in percentages on the y-axis. (B) Summary of clonality assessment detecting relapsed cHL in 24 patients, together with EBV status, cHL subtype, and time interval between the paired cHL samples. CT, clonality threshold; D, primary diagnosis sample; NOS, not otherwise specified; R, recurrence sample; y, years.

patients [79%]). Confirmation of clonal relationship was based on identical clonotypes for at least 2 distinct IG rearrangement targets in 13 of 24 patients (54%), whereas for the remaining patients, this involved a single IG target (supplemental Table 7). In 9 of 24 patients with relapsed cHL, the clonal relationship was based on identical FR2/FR3 IGHV-IGHD-IGHJ gene rearrangements. Their complementarity-determining region 3 (CDR3) displayed nucleotide sequence differences with the germ line sequence, indicative

of somatic hypermutation (SHM). However, comparison between primary diagnosis and relapsed disease revealed identical sequences without any additional nucleotide substitutions, even after ~11 years (supplemental Table 8). This demonstrates a lack of ongoing SHM between primary diagnosis and relapsed cHL. Furthermore, 4 patients (cases 6, 9, 40, and 56) showed additional unique clonal IG gene rearrangement(s) besides the shared clonotype(s) in either diagnosis or recurrence (supplemental Table 7). These additional clonotypes, which were present at multiple IG targets for 3 patients, displayed a distinct dominance compared with the shared clonotype(s), indicative for the presence of a second B-cell clone within these tissue samples.

IG-NGS clonality analysis demonstrates clonally unrelated lymphomas in cHL recurrences

Besides the 24 clonally related cHL recurrences, 5 of 53 patients (cases 4, 5, 8, 14, and 43) showed distinct clonal IG gene rearrangements (termed as distinct rearrangements) in the paired samples, indicating the presence of different B-cell clones and clonally unrelated cHL (Figure 2; supplemental Table 9A). In another 5 patients (cases 7, 19, 21, 52, and 54), specific clonal IG gene rearrangements were detected in either diagnosis or recurrence, whereas these dominant clonotypes were nondetectable in the reciprocal samples (termed as discordant rearrangements; supplemental Table 9B). Genomic DNA quality, HRS cell percentage (as a fraction of total B cells), and the lack of ongoing SHM between diagnosis and recurrence were all parameters that could not explain the inability of detecting these clonotypes in the complete data set for IGHV-IGHD-IGHJ (range clonotypes, 683-3168), IGKV-IGKJ (range clonotypes, 720-1036), and IGKV/Intron-KDE (range clonotypes, 166-678) rearrangements of the reciprocal samples (supplemental Table 10; supplemental Figure 2). This indicated that the paired sample lacked the malignant B-cell clone detected in the reciprocal sample and, instead, harbored a clone that could not be identified with the standard IG-NGS assay, as was the case for the 37 cHL samples of the 19 patients that remained inconclusive for clonal comparison. Therefore, 10 cases were classified as clonally unrelated (Figure 2; supplemental Table 9B).

In the total cohort of clonally unrelated cHL, EBV positivity was present in 4 of 10 diagnosis samples (40%), including 2 MCHL (cases 19 and 54) and 2 NSHL subtypes (cases 5 and 8). This rate of EBV positivity was higher than cHL diagnosis in the clonally confirmed relapse cohort (n = 3 of 24, 13%; $P = .071$). Notably, the EBV status switched between primary diagnosis and second cHL in 3 of 10 clonally unrelated patients (cases 7, 8, and 14). The clonally unrelated cHL recurrences occurred in all age groups, and 7 of 10 patients (70%) showed a time to recurrence of >2 years (range, 2.8-13.0 years), compared with 5 of 24 patients (21%) with a confirmed relapse (range, 2.1-4.0 years; $P = .006$).

Altogether, the IG-NGS clonality data demonstrate that, in this study cohort, the clonal relationship could be established in 34 of 53 patients (64%) by NGS-based detection of IG gene rearrangements; of which 24 of 34 patients (71%) developed relapsed disease and 10 of 34 patients (29%) displayed a clonally unrelated de novo cHL. In the remaining 19 patients, the absence of detectable clonal IG gene rearrangements in whole tissue specimens of diagnosis and/or recurrence resulted in inconclusive results (Figure 3).

Mutation analysis confirms the occurrence of second primary cHL

As demonstrated by NGS-based Ig clonality assessment, 10 patients were suspected of a second primary cHL. To further substantiate these results, targeted mutation analysis on whole tissue specimen of the paired diagnosis and recurrence samples (n = 10) was performed with TSO500 assay (600 × average median exon coverage) (supplemental Table 11). This hybrid capture panel harbors different cancer-related genes, including 100 lymphoma-associated, but not cHL-specific, target genes (supplemental Table 5A). The detection of single nucleotide polymorphisms (SNPs) reminiscent of germ line variants confirmed that all sample pairs were from the same patient (average, 5 SNPs; range, 2-9 SNPs; data not shown).

Next, (potential) pathogenic mutations (PA3-PA5) with a variant allele frequency (VAF) from 1% to 30%, were selected to identify candidate HRS-specific gene mutations and compared between paired diagnosis and recurrence sample. In 4 of 5 patients with distinct rearrangements in diagnosis and recurrence, mutually exclusive gene mutations were identified in both samples, except for 1 overlapping *TET2* mutation in case 5 (p.Q731Tfs*22; VAF diagnosis, 3%; VAF recurrence, 14%; Figure 4A; supplemental Table 11A). The mutually exclusive mutations represented known cHL target genes, including *ARID1A*, *B2M*, *CD274*, *EP300*, *GNA13*, *SMARCA4*, *SOCS1*, *SPEN*, *STAT5A*, and *TNFAIP3* as well as other cancer-related genes (Figure 4A-B; supplemental Table 11A). Similarly, 4 of 5 patients with discordant rearrangements harbored mutually exclusive mutations in diagnosis and recurrence (eg, *ARID1A*, *ARID1B*, *DOT1L*, *GNA13*, *SPEN*, and *TNFAIP3*). Here, 3 patients showed single overlapping mutations between diagnosis and recurrence, which included 2 patients harboring a *B2M* mutation (case 21, p.V9G and case 52, p.M1R) with a VAF ranging from 2% to 7% (Figure 4C) and 1 patient (case 54) showing a *H1-2* mutation (p.I80Afs*11; VAF diagnosis and recurrence, 2%; supplemental Table 11B). No pathogenic mutations were detected with TSO500 assay in both diagnosis and recurrence for cases 14 and 19. Together, these targeted mutation analyses supported the identification of clonally unrelated cHL in this study cohort and the occurrence of second primary cHL.

The clinical data for most of these patients revealed no underlying disease explaining the occurrence of such second primary cHL. However, 1 patient (case 19) showed a complex clinical history, with chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) syndrome 6 years after the cHL recurrence and autoimmune hemolytic anemia. This suggested an underlying history of an immune dysregulating disorder that might have contributed to the development of 2 independent lymphomas. Another patient (case 54) with a positive family history of cHL developed cHL twice before the age of 9 years together with serological and molecular evidence for chronic active EBV infection, also suggesting an underlying genetic defect.

Detection of clonal TR gene rearrangements and TCL-associated mutations in cHL

In 43% of the cHL samples, no clonal IG gene rearrangements were detected. Because clonal TR gene rearrangements have been described in cHL, TR-NGS clonality assessment was performed in 57 paired cHL diagnosis and recurrences for which

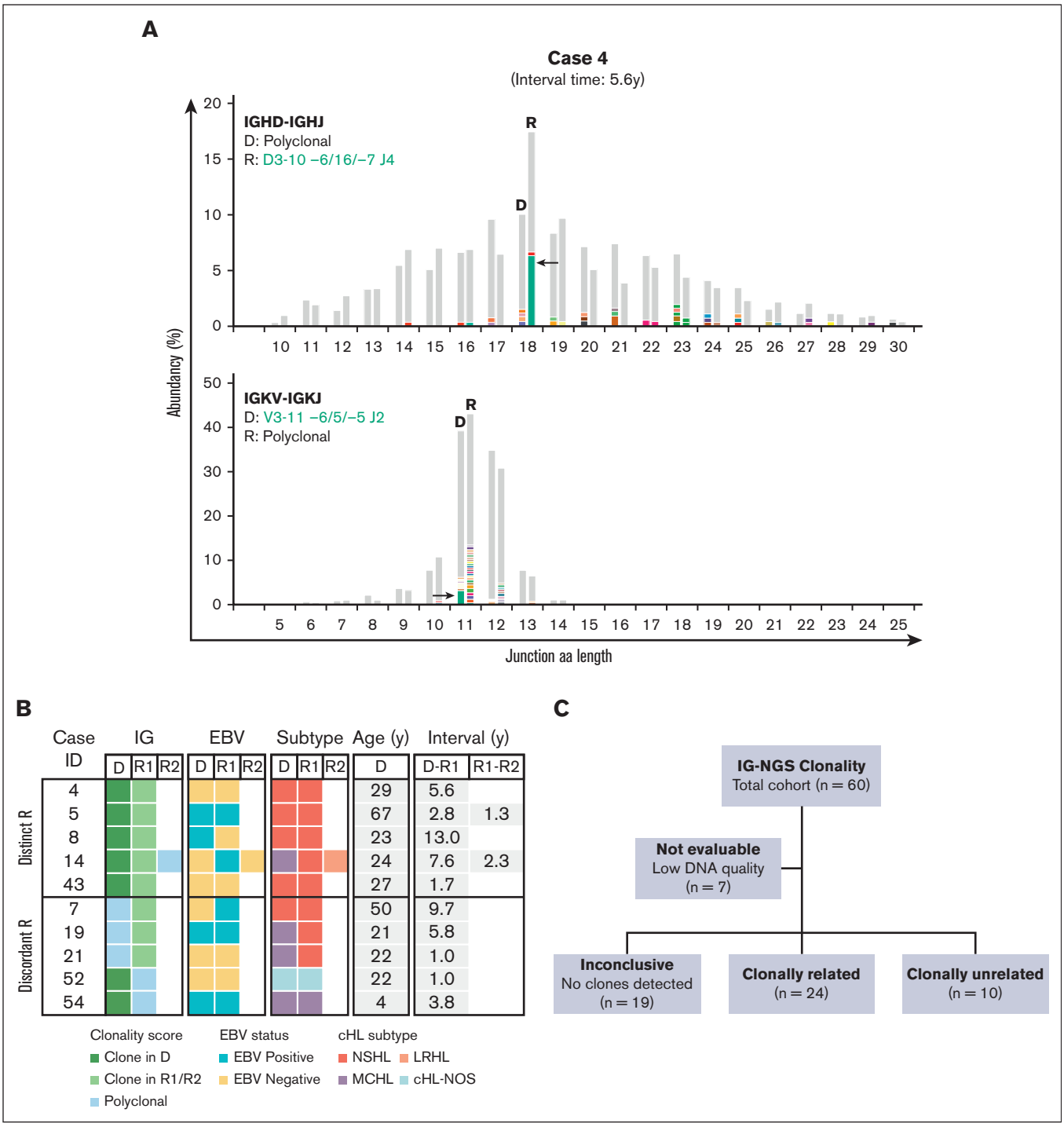

Figure 2. NGS-based clonality analysis identifies clonally unrelated second primary cHL recurrences. (A) Representative data sets indicating NGS-based detection of IG gene rearrangements in paired diagnosis (D) and recurrence (R) tissue samples of case 4. Data of 2 IG targets (IGHD-IGHJ and IGKV-IGKJ) are shown. The specific clonotypes for the dominant clonal IG gene rearrangement are indicated in green. On the x-axis, the junction aa length is shown, and the abundancy of clonotypes is shown in percentages on the y-axis. (B) Summary of clonality assessment detecting second primary cHL in 10 patients. Two groups of clonally unrelated cases were defined, based on the detection of distinct clonal IG gene rearrangement(s) (distinct R) or clonal IG gene rearrangement(s) in 1 sample absent in the paired sample (discordant R). EBV status, cHL subtype, and time interval between the paired cHL samples are indicated. (C) Schematic overview of the clonal relationship of paired diagnosis and recurrence samples of the complete diagnosis and recurrence cHL cohort, based on IG-NGS clonality assessment.

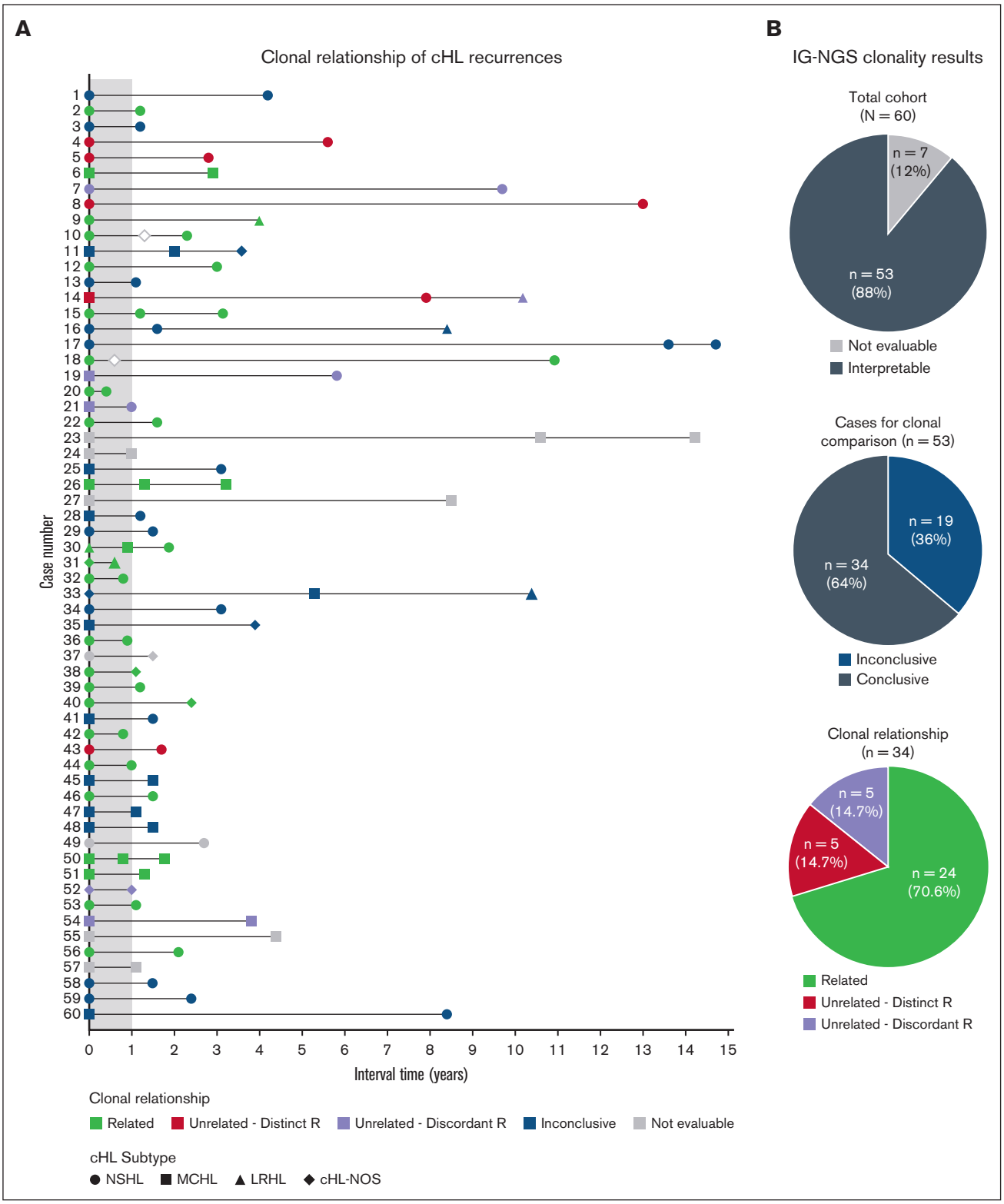

Figure 3. Summary clonal relationship of paired diagnosis and recurrence cHL samples. (A) Clonal comparison revealed the clonal relationship of paired diagnosis and recurrence samples by NGS-based detection of immunoglobulin rearrangements (IG-NGS). Time interval between the diagnoses and the cHL subtype of each biopsy is indicated. The open diamonds represent recurrences for which no tissue biopsy was available; therefore, molecular analysis could not be performed. (B) The top pie chart shows the proportion of cases with interpretable and nonevaluable data sets within the total cohort, the middle pie chart the proportion of cases with conclusive and inconclusive data sets, and the bottom pie chart the clonal relationship of all cases with conclusive results. Clonally unrelated cHL is subdivided in cases with distinct R or discordant R.

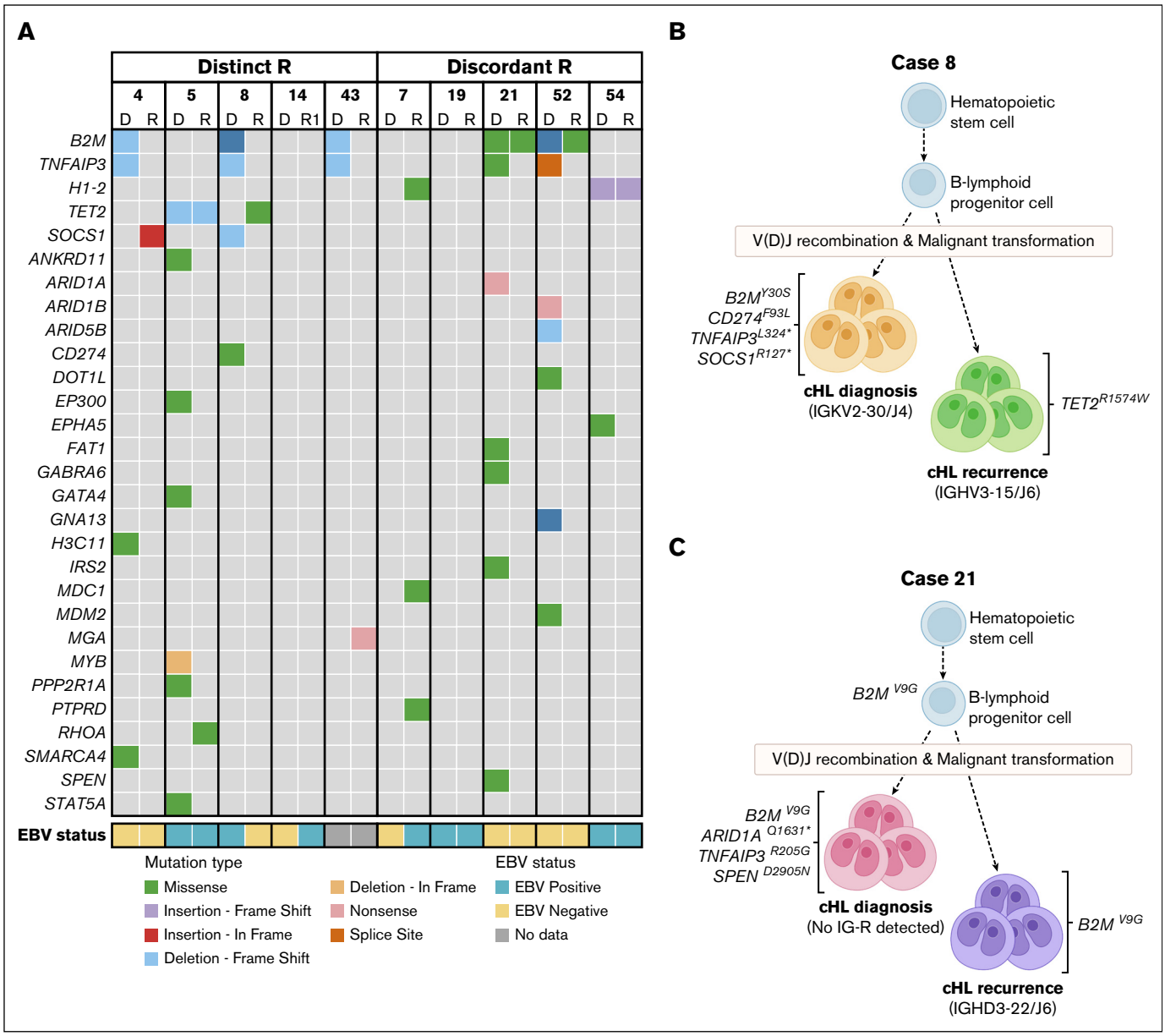

Figure 4. Mutation analysis supports the identification of second primary lymphoma in cHL recurrences. Tissues of paired diagnosis and recurrences representing second primary cHL were subjected to targeted mutation analysis (TSO500 assay). HRS-associated gene mutations were selected based on a VAF of 1% to 30% and a minimal coverage of 350 reads. (A) Left panel shows data of clonally unrelated cHL samples based on distinct R, and right panel shows data of cases with discordant R. Paired data show mutually exclusive mutations and single overlapping mutations for 8 cases, whereas tissue samples of 2 cases (14 and 19) lacked pathogenic gene mutations with TSO500 assay. Mutation types and EBV status are shown. All shown mutations are scored as (potentially) pathogenic (PA3-PA5). For detailed information, see supplemental Table 9. (B-C) Schematic representations of the development of 2 independent lymphomas of case 8 (panel B) and case 21 (panel C). Gene mutations associated with cHL and clonal Ig rearrangement targets are indicated.

sufficient genomic DNA was available (n = 125 samples). After defining thresholds for T-cell clonality in cHL (supplemental Information), 14 of 125 samples (11%) displayed clonal TRB/TRG gene rearrangements, whereas the remaining samples showed a polyclonal pattern (supplemental Table 12). In 5 of 14 samples, both clonal IG and TR gene rearrangements were present (Figure 5). Overall, in 12 of 57 patients (21%), clonal TR gene rearrangements were detected in diagnosis (n = 5) and/or recurrence (n = 9). Four patients (cases 1, 5, 11, and 23) showed preserved dominant TCR clonotypes in paired diagnosis and relapse (supplemental Table 13). To assess whether detection of TCR clonality was associated with cHL relapse, TR-NGS was performed in a control cohort of patients with cHL without relapse (n = 48). Here, 3 of 48 samples (6%) displayed clonal TR gene rearrangements (supplemental Table 12), indicating that TCR clonality as such is not unique for relapse-prone cHL.

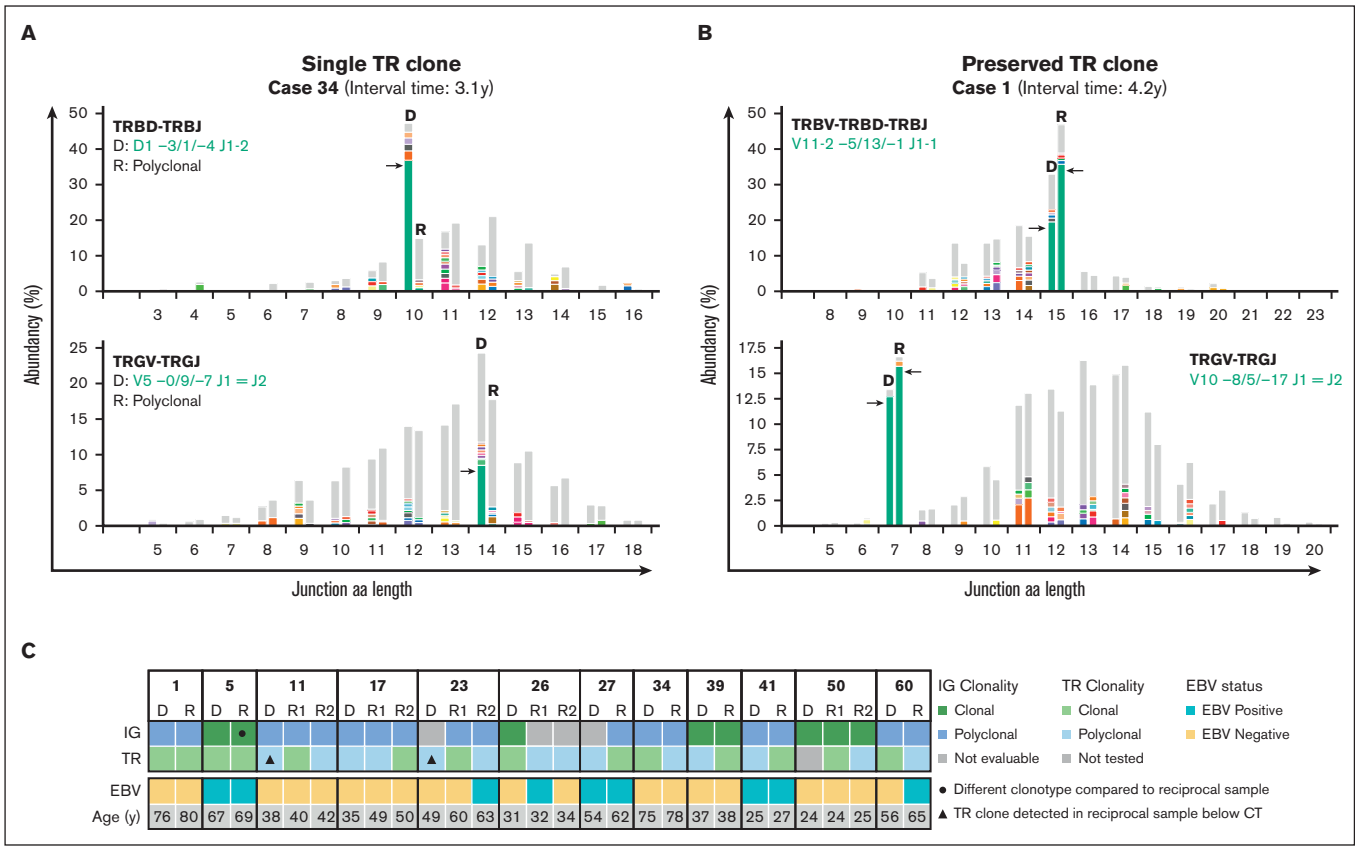

Figure 5. NGS-based T-cell clonality analysis identifies clonal TR gene rearrangements in cHL samples. (A) Representative data set indicating NGS-based detection of TR gene rearrangements in paired diagnosis (D) and recurrence (R) tissue samples of a case with a dominant T-cell clone in only the primary diagnosis but not recurrence (case 34). The results of 2 TCR targets (TRBV-TRBD-TRBJ and TRGV-TRGJ) are shown, and the specific clonotypes for the dominant TR gene rearrangements are indicated. On the x-axis, the junction aa length is shown, and the abundancy of clonotypes is shown in percentages on the y-axis. (B) Representative data set indicating NGS-based detection of TR gene rearrangements in paired samples with a preserved identical dominant T-cell clone (case 1). (C) Summary of IG- and TR-NGS clonality results of all patients with at least 1 sample with a dominant TCR clone. EBV status of all tumor samples and age of the patients are shown.

To determine whether cHL samples with clonal TR gene rearrangement were instead TCLs mimicking cHL, mutation analysis was performed for TCL-related genes. First, potential AITL-associated mutations in *DNMT3A*, *IDH2*, *RHOA*, and *TET2* were investigated in 13 samples with a clonal TR gene rearrangement by a targeted NGS approach, as previously described.[32] Here, 7 samples showed *TET2* mutations with a VAF ranging from 3% to 50%, which potentially also included CHIP-related mutations (supplemental Table 14). Furthermore, in 3 of 7 *TET2*-mutated samples, the *RHOA* hotspot mutation p.G17V was detected. Second, a selected set of 6 samples with a dominant TCR clone were analyzed with a lymphoma-specific 200 gene panel (BLYMF200)[42] to identify other TCL-associated mutations. Here, all 6 samples from 5 patients showed TCL-associated gene mutations, including *DNMT3A* (case 60, p.E856G), *STAT3* (case 1, p.S614R and case 11, p.D661Y), *VAV1* (case 27, p.M501V), and *JAK3* (case 1, p.I239T and case 27, p.T435A; supplemental Table 14).

These molecular findings were indicative of TCL mimicking cHL, and candidate tissue samples were reassessed by pathology review. One patient (case 1), who showed preserved TCR clonotypes in paired diagnosis and relapse samples, exhibited *STAT3* and *JAK3* mutations, along with large lymphoid cells strongly positive for CD30 but negative for PAX5 and anaplastic lymphoma kinase (ALK). This case was reclassified as ALK–negative anaplastic large cell lymphoma (ALCL). Recurrence samples of 3 other patients (cases 5, 23, and 27) showed *RHOA* and *TET2* mutations and immunohistology in line with AITL mimicking cHL (Figure 6; supplemental Figure 3). Two primary diagnosis samples displayed composite cHL and AITL (cases 5 and 60), based on molecular results (IG/TR clonality and gene mutations) and histomorphology. The recurrence of case 11 harboring a dominant T-cell clone and *STAT3* mutation was still considered a cHL. Altogether, in 5 patients (n = 7 samples), retrospective pathology review with integration of the molecular findings indicated the presence of a TCL, of which the HRS-like cells in 3 AITL samples of 2 patients showed EBV-positive results.

Discussion

Investigating the clonal relationship of lymphoma recurrences reveals the occurrence of a second primary lymphoma. In our study, NGS-based detection of IG gene rearrangements showed clonally related relapsed cHL in approximately two-third of patients with cHL, whereas clonally unrelated second primary cHL was detected

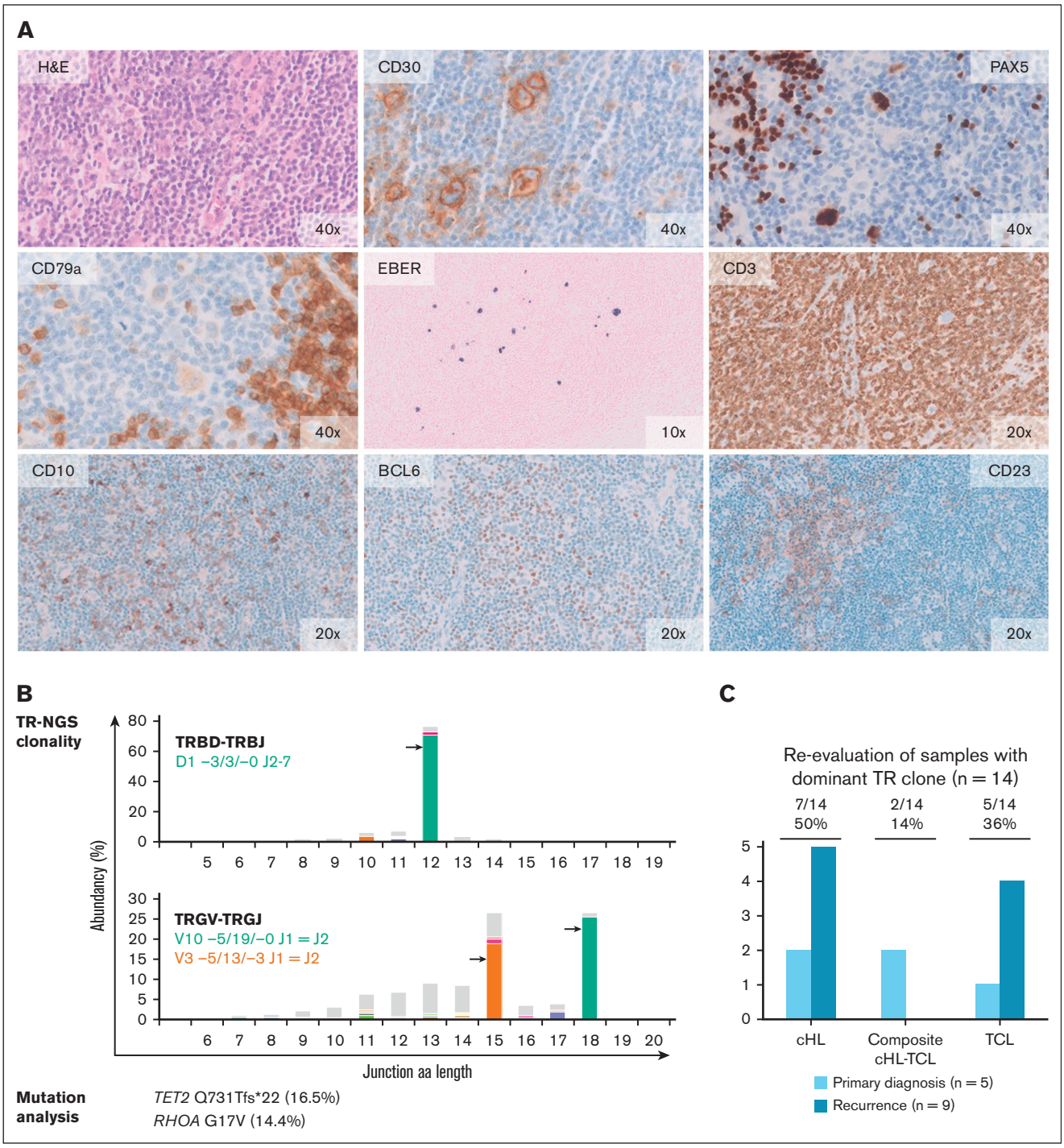

Figure 6. Molecular analysis and histomorphological reevaluation reveal AITL diagnosis in cHL recurrence. Histomorphology and molecular data of the recurrence sample of case 5. (A) The normal lymph node architecture has been replaced by a polymorphous infiltrate consisting of small- and medium-sized lymphocytes with scattered cells having large nuclei and prominent nucleoli, some of which are consistent with HRS-like cells. High-endothelial venules are prominent. The large cells show strong expressions of CD30 and PAX5 and a weak expression of CD79a. Epstein-Barr encoding region (EBER) in situ hybridization shows scattered positive cells of different size. Anti-CD3 staining reveals numerous small- to medium-sized T cells. Many of these cells are positive for programmed cell death protein 1 (PD1) (data not shown) and a small proportion shows expression of CD10 and BCL6. Anti-CD23 staining demonstrates slight expansion of irregular dendritic meshworks. The original magnification (×10, ×20 and ×40) is indicated. (B) TR-NGS clonality analysis shows the presence of clonal TRBD- TRBJ and TRGV- TRGJ rearrangements. Targeted mutation analysis reveals mutations in AITL-associated genes *TET2* and *RHOA*; VAF is indicated. (C) Summary of morphological reevaluation of the samples with a dominant TCR clone with integration of molecular findings. In total, 7 samples of 5 patients were indicative for TCL.

in almost one-third of patients with cHL. T-cell clonality analysis demonstrated clonal TR gene rearrangements in ~10% of the assumed cHL tissue samples. Combined with the identification of TCL-related gene mutations, this led retrospectively to TCL with HRS-like cells mimicking cHL diagnosis in ~50% of the samples harboring a clonal TR gene rearrangement.

Considering all archival tissues analyzed in this study, IG-NGS clonality assessment detected B-cell clonality in 58% of cHL samples, which is similar to our previous findings in primary cHL whole tissue specimens.[16] This allowed for the clonal comparison of primary diagnosis and recurrence in 34 patients with cHL, revealing clonally related relapsed cHL disease in 24 patients, with a median time to relapse of 1.2 years (range, 0.4-4 years). Notably, in 4 clonally related samples, a second B-cell clone was identified with a different abundancy compared with the preserved neoplastic clonotypes. We considered this suggestive of either biclonal cHL, as demonstrated previously,[16] or the presence of a reactive B-cell clone.

The occurrence of clonally unrelated second primary cHL was based on the detection of distinct rearrangements in 5 paired primary diagnosis and recurrences. For another 5 patients with cHL, the identified clonal IG gene rearrangements were completely absent in the reciprocal polyclonal sample, representing discordant rearrangements. Because these samples harbored good quality genomic DNA with deep coverage NGS data sets and were considered negative for ongoing SHM between diagnosis and recurrence, the latter 5 cases were also considered clonally unrelated cHL. Thus, IG clonality assessment indicated the occurrence of a second primary cHL in a total of 10 patients. Targeted mutation analysis confirmed these findings in 8 patients (4 patients in each group) by the presence of mutually exclusive mutations in the paired primary diagnosis and recurrence samples, whereas 2 patients showed no detectable somatic mutations with the targeted gene panel. The pathogenic cHL-associated gene alterations included *B2M*, *SOCS1*, and *TNFAIP3* loss-of-function mutations but also other cancer-associated genes, such as *MGA*.

Besides the presence of these mutually exclusive mutations, single mutations were identified that were shared between diagnosis and recurrence in 4 patients with clonally unrelated cHL. In 1 patient (case 5), this involved a *TET2* mutation combined with a *RHOA* hotspot mutation (only detected above threshold in recurrence) related to a minor AITL component in diagnosis and AITL in recurrence. In the other 3 patients (age, 4-23 years), these represented mutations in *B2M* (2 patients) and *H1-2* (one patient), with a VAF <7%. We propose that these latter shared mutations might have occurred in an ancestor cell at an early stage of B-cell development (ie, before IG gene rearrangement), and via branching, clonal evolution resulted into 2 independent consecutive cHL tumors. Our findings by IG-NGS of clonally unrelated second primary cHL in approximately one-third of the patients is in line with a previous report detecting clonally unrelated cHL in 8 of 20 patients (40%) with cHL.[37] Similar to their observation, we observed a switch in EBV-positivity status between primary diagnosis and recurrence in 3 of 10 patients, which seldomly occurs in clonally related relapsed cHL.

The occurrence of second de novo lymphomas might be linked to an immune dysregulation disorder or lymphoma predisposition. Monozygotic twins of patients with cHL display a highly increased risk (~100-fold) of developing cHL and relatives of patients with cHL also show increased risk of cHL.[44,45] Based on genome wide association studies, certain HLA alleles result in an increased susceptibility for cHL,[46] whereas other HLA alleles act as a protective factor for cHL development.[47-49] SNPs identified as cHL risk factors are often present in genes related to immunity, such as *BMF*, *GATA3*, *IRF4*, *PAX5*, *STAT3*, *TCF3*, *TLR4*, and *TP63*.[50-55] Furthermore, specific cHL predisposing genes have been reported, including *CTLA4*, *DICER1*, *MKL1*, *ACAN*, and *KDR*.[54,56-60] Because at least 2 patients in this study cohort with second primary cHL were linked to immune dysregulation and/or genetic predisposition, future studies using whole genome sequencing might reveal whether specific germ line variants are affected in these patients.

By using IG/TR-NGS clonality assessment combined with targeted mutation analysis, we were able to reevaluate the initial diagnosis of several patients. Tissue biopsy specimen of 5 patients with a dominant TCR clone and TCL-associated mutations were retrospectively diagnosed as TCL instead of cHL. The primary diagnosis of samples of 2 of 5 patients were likely composite lymphomas consisting of cHL and a (minor) TCL component. Dominant TR gene rearrangements in the other patients were considered to represent reactive T-cell clones, because there was no evidence for TCL-associated mutations or histomorphology. In fact, minor T-cell clones (below the clonality threshold of 5%) were also detected in other cHL samples (data not shown). Such T-cell clones probably represent clonal expansions of reactive T cells, for instance, against EBV^+ HRS cells with detectable major histocompatibility complex class I and/or class II expression.[61] Our findings of misdiagnosed cHL is in line with a recent study in which 18 of 54 selected patients with cHL were reclassified as TCL.[62] Indeed, TCL such as angioimmunoblastic type nTFHL/AITL are increasingly recognized as a diagnostic pitfall in the context of cHL. Therefore, TCR clonality assessment and/or targeted TCL-associated gene mutation analysis should be considered in routine diagnostics to avoid misdiagnosis in cases with morphological or clinical doubt and in cases with disease recurrence.

Finally, our mutation analyses in patient samples with a dominant TCR clone also revealed *TET2* mutations with VAFs of >20% in 4 of 11 patients (36%), which did not correlate with the percentage of neoplastic HRS cells within the cHL tissue specimens. This possibly reflected CHIP-associated mutations that may also reside within HRS cells themselves, as demonstrated recently.[36] The relative high frequency of such variants in this subset of predominantly older patients with cHL (>50 years) with disease recurrence in combination with an underlying TCL suggests a role for these CHIP-associated mutations in lymphoma development, such as in other hematopoietic neoplasms.[63-65]

In conclusion, our in-depth molecular analysis demonstrates the occurrence of second de novo cHL in approximately one-third of patients with cHL and ~60% of patients with cHL, with a time to recurrence of >2 years after primary cHL diagnosis. Furthermore, our data show that in retrospect 5 other patients were diagnosed with TCL. These findings obviously have strong clinical implications, because these patients should receive different treatment strategies. The outcome of our study indicates that NGS-based clonality assessment combined with targeted mutation analysis should be considered in routine diagnostics for patients with cHL with recurrences after 2 years.

Acknowledgments

The authors thank Karin Beunen and Harald Verheij (Rijnstate Hospital) and Lieneke Homans-ter Keurs (Gelderse Vallei Hospital) for their assistance in gathering clinical data, supporting staff for collecting tumor samples from pathology archives, Maria Pafiti for DNA isolation and clonality assessment of the nonrelapse control cohort, and the technical staff of the departments of pathology and human genetics for preparing samples for TSO500 assay and loading NGS runs. Panels B and C of Figure 4 were created with BioRender.com.

This work was funded by the Dutch Cancer Society (KWF-11137) and the Dutch Health Insurers' Innovation Fund (project number 17-179).

Authorship

Contribution: J.H.J.M.v.K. and B.S. designed the research and conceived the project; D.A.G.v.B., J.L.M.v.d.L.-K., J.R., J.A.C.W.L., I.B., R.A.L.d.G., and F.A.d.G. performed the experimental research; D.A.G.v.B., L.I.K., M.R.B., P.J.T.A.G., K.M.H., M.v.d.B., I.B., J.S.P.V., R.A.L.d.G., and B.S. were involved in data analysis and interpretation; D.A.G.v.B., W.B.C.S., E.v.d.S., W.J.P., J.F.M.P., S.D.P.W.M.d.J.-P., G.A.V., C.L., E.R.v.B., B.F., B.M.H., A.P., J.v.d.W.t.B., P.T.G.A.N., K.M.H., F.F., A.D., and M.v.d.B. collected the histological material and/or clinical data; D.A.G.v.B. and B.S. wrote the manuscript; M.v.d.B., J.H.J.M.v.K., K.M.H., and A.D. examined histopathology; and all authors read and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: D.A.G.v.B., 0000-0002-4289-2807; L.I.K., 0000-0002-2666-1742; J.L.M.v.d.L.-K., 0000-0003-0191-9935; W.J.P., 0000-0001-9828-9460; A.P., 0000-0003-3089-6947; J.S.P.V., 0000-0002-1628-6256; K.M.H., 0000-0002-4181-3302; A.D., 0000-0001-9239-1050; P.J.T.A.G., 0000-0003-4314-228X; B.S., 0000-0001-8029-9230.

Correspondence: Blanca Scheijen, Pathology, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands; email: blanca.scheijen@radboudumc.nl.

References

1. Shanbhag S, Ambinder RF. Hodgkin lymphoma: a review and update on recent progress. *CA Cancer J Clin*. 2018;68(2):116-132.
2. Swerdlow SH, Campo E, Harris NL, Pileri SA. *WHO classification of tumours of haematopoietic and lymphoid tissues*. International Agency for Research on Cancer; 2017.
3. André MPE, Girinsky T, Federico M, et al. early positron emission tomography response-adapted treatment in stage I and II Hodgkin lymphoma: final results of the randomized EORTC/LYSA/FIL H10 trial. *J Clin Oncol*. 2017;35(16):1786-1794.
4. Borchmann P, Goergen H, Kobe C, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin Study Group. *Lancet*. 2017;390(10114):2790-2802.
5. Carde P, Karrasch M, Fortpied C, et al. Eight cycles of ABVD versus four cycles of BEACOPPescalated plus four cycles of BEACOPPbaseline in stage III to IV, international prognostic score ≥3, high-risk Hodgkin lymphoma: first results of the phase III EORTC 20012 intergroup trial. *J Clin Oncol*. 2016;34(17):2028-2036.
6. Montanari F, Diefenbach C. Relapsed Hodgkin lymphoma: management strategies. *Curr hematol malig rep*. 2014;9(3):284-293.
7. Biccler JL, Glimelius I, Eloranta S, et al. Relapse risk and loss of lifetime after modern combined modality treatment of young patients with Hodgkin lymphoma: a Nordic lymphoma epidemiology group study. *J Clin Oncol*. 2019;37(9):703-713.
8. Hapgood G, Zheng Y, Sehn LH, et al. Evaluation of the risk of relapse in classical Hodgkin lymphoma at event-free survival time points and survival comparison with the general population in British Columbia. *J Clin Oncol*. 2016;34(21):2493-2500.
9. Bröckelmann PJ, Goergen H, Kohnhorst C, et al. Late relapse of classical hodgkin lymphoma: an analysis of the German Hodgkin study group HD7 to HD12 trials. *J Clin Oncol*. 2017;35(13):1444-1450.
10. Vassilakopoulos TP, Kravvariti E, Panitsas F, et al. Very late relapses in Hodgkin lymphoma treated with chemotherapy with or without radiotherapy: linear pattern and distinct prognostic factors. *Blood Cancer J*. 2022;12(7):102-106.
11. Andersen MD, Hamilton-Dutoit S, Modvig L, et al. Late recurrence of lymphoid malignancies after initial treatment for Hodgkin lymphoma - a study from the Danish Lymphoma Registry. *Br J Haematol*. 2022;198(1):50-61.
12. Marafioti T, Hummel M, Foss HD, et al. Hodgkin and reed-sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood*. 2000;95(4):1443-1450.
13. Kuppers R, Rajewsky K, Zhao M, et al. Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *Proc Natl Acad Sci U S A*. 1994;91(23):10962-10966.
14. 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.
15. van den Brand M, Rijntjes J, Mobs 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.
16. 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.

17. Hartmann S, Helling A, Doring C, Renne C, Hansmann ML. Clonality testing of malignant lymphomas with the BIOMED-2 primers in a large cohort of 1969 primary and consultant biopsies. *Pathol Res Pract*. 2013;209(8):495-502.
18. Nguyen TT, Warnke RA, Seo K, Rosenberg SA, Arber DA. Rare presentation of classical Hodgkin lymphoma with a clonal T-cell receptor gene rearrangement in the tissue. *Leuk Lymphoma*. 2010;51(7):1356-1359.
19. Aguilera NS, Chen J, Bijwaard KE, et al. Gene rearrangement and comparative genomic hybridization studies of classic Hodgkin lymphoma expressing T-cell antigens. *Arch Pathol Lab Med*. 2006;130(12):1772-1779.
20. Muschen M, Rajewsky K, Brauninger A, et al. Rare occurrence of classical Hodgkin's disease as a T cell lymphoma. *J Exp Med*. 2000;191(2):387-394.
21. Seitz V, Hummel M, Marafioti T, Anagnostopoulos I, Assaf C, Stein H. Detection of clonal T-cell receptor gamma-chain gene rearrangements in Reed-Sternberg cells of classic Hodgkin disease. *Blood*. 2000;95(10):3020-3024.
22. Tzankov A, Bourgau C, Kaiser A, et al. Rare expression of T-cell markers in classical Hodgkin's lymphoma. *Mod Pathol*. 2005;18(12):1542-1549.
23. Willenbrock K, Ichinohasama R, Kadin ME, et al. T-cell variant of classical Hodgkin's lymphoma with nodal and cutaneous manifestations demonstrated by single-cell polymerase chain reaction. *Lab Invest*. 2002;82(9):1103-1109.
24. Huang W, Xie J, Xu X, Gao X, Xie P, Zhou X. MUM-1 expression differentiates AITL with HRS-like cells from cHL. *Int J Clin Exp Pathol*. 2015;8(9):11372-11378.
25. Parente P, Zanelli M, Sanguedolce F, Mastracci L, Graziano P. Hodgkin Reed-Sternberg-like cells in non-Hodgkin lymphoma. *Diagnostics (Basel)*. 2020;10(12):1019-1048.
26. Xie Y, Jaffe ES. How I diagnose angioimmunoblastic T-cell lymphoma. *Am J Clin Pathol*. 2021;156(1):1-14.
27. Gomez-Gelvez JC, Smith LB. Reed-Sternberg-like cells in non-Hodgkin lymphomas. *Arch Pathol Lab Med*. 2015;139(10):1205-1210.
28. Moroch J, Copie-Bergman C, de Leval L, et al. Follicular peripheral T-cell lymphoma expands the spectrum of classical Hodgkin lymphoma mimics. *Am J Surg Pathol*. 2012;36(11):1636-1646.
29. Brune MM, Rau A, Overkamp M, et al. Genomic landscape of Hodgkin lymphoma. *Cancers (Basel)*. 2021;13(22):5605-5618.
30. van Bladel DAG, Stevens WBC, van den Brand M, et al. Novel approaches in molecular characterization of classical Hodgkin lymphoma. *Cancers (Basel)*. 2022;14(13):3222-3237.
31. Weniger MA, Küppers R. Molecular biology of Hodgkin lymphoma. *Leukemia*. 2021;35(4):968-981.
32. Steinhilber J, Mederake M, Bonzheim I, et al. The pathological features of angioimmunoblastic T-cell lymphomas with IDH2(R172) mutations. *Mod Pathol*. 2019;32(8):1123-1134.
33. Odejide O, Weigert O, Lane AA, et al. A targeted mutational landscape of angioimmunoblastic T-cell lymphoma. *Blood*. 2014;123(9):1293-1296.
34. Challen GA, Goodell MA. Clonal hematopoiesis: mechanisms driving dominance of stem cell clones. *Blood*. 2020;136(14):1590-1598.
35. Xie Z, Zeidan AM. CHIPing away the progression potential of CHIP: a new reality in the making. *Blood Rev*. 2023;58:101001-101018.
36. Venanzi A, Marra A, Schiavoni G, et al. Dissecting clonal hematopoiesis in tissues of classical Hodgkin lymphoma patients. *Blood Cancer Discov*. 2021;2(3):216-225.
37. Obermann EC, Mueller N, Rufle A, et al. Clonal relationship of classical hodgkin lymphoma and its recurrences. *Clin Cancer Res*. 2011;17(16):5268-5274.
38. Bruggemann M, Kotrova 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.
39. van Bladel DAG, van der Last-Kempkes JLM, Scheijen B, Groenen P; EuroClonality Consortium. Next-generation sequencing-based clonality detection of immunoglobulin gene rearrangements in B-cell lymphoma. *Methods Mol Biol*. 2022;2453:7-42.
40. Bystry V, Reigl T, Krejci A, et al. ARResT/interrogate: an interactive immunoprofiler for IG/TR NGS data. *Bioinformatics*. 2017;33(3):435-437.
41. Kroeze LI, de Voer RM, Kamping EJ, et al. Evaluation of a hybrid capture-based pan-cancer panel for analysis of treatment stratifying oncogenic aberrations and processes. *J Mol Diagn*. 2020;22(6):757-769.
42. de Groot FA, de Haan LM, de Groen RAL, et al. Synchronous diffuse large B-cell lymphoma and mantle cell lymphoma: support for low-threshold biopsies and genetic testing. *Leuk Lymphoma*. 2022;63(5):1251-1255.
43. Brice P, de Kerviler E, Friedberg JW. Classical Hodgkin lymphoma. *Lancet*. 2021;398(10310):1518-1527.
44. Goldin LR, Pfeiffer RM, Gridley G, et al. Familial aggregation of Hodgkin lymphoma and related tumors. *Cancer*. 2004;100(9):1902-1908.
45. Mack TM, Cozen W, Shibata DK, et al. Concordance for Hodgkin's disease in identical twins suggesting genetic susceptibility to the young-adult form of the disease. *N Engl J Med*. 1995;332(7):413-418.
46. Kushekhar K, van den Berg A, Nolte I, Hepkema B, Visser L, Diepstra A. Genetic associations in classical hodgkin lymphoma: a systematic review and insights into susceptibility mechanisms. *Cancer Epidemiol Biomarkers Prev*. 2014;23(12):2737-2747.
47. Hjalgrim H, Rostgaard K, Johnson PC, et al. HLA-A alleles and infectious mononucleosis suggest a critical role for cytotoxic T-cell response in EBV-related Hodgkin lymphoma. *Proc Natl Acad Sci U S A*. 2010;107(14):6400-6405.
48. Huang X, Kushekhar K, Nolte I, et al. HLA associations in classical Hodgkin lymphoma: EBV status matters. *PLoS One*. 2012;7(7):e39986-39994.
49. Niens M, Jarrett RF, Hepkema B, et al. HLA-A*02 is associated with a reduced risk and HLA-A*01 with an increased risk of developing EBV+ Hodgkin lymphoma. *Blood*. 2007;110(9):3310-3315.

50. Broderick P, Cunningham D, Vijayakrishnan J, et al. IRF4 polymorphism rs872071 and risk of Hodgkin lymphoma. *Br J Haematol*. 2010;148(3):413-415.
51. Butterbach K, Beckmann L, de Sanjosé S, et al. Association of JAK-STAT pathway related genes with lymphoma risk: results of a European case-control study (EpiLymph). *Br J Haematol*. 2011;153(3):318-333.
52. Monroy CM, Cortes AC, Lopez MS, et al. Hodgkin disease risk: role of genetic polymorphisms and gene-gene interactions in inflammation pathway genes. *Mol Carcinog*. 2011;50(1):36-46.
53. Nieters A, Beckmann L, Deeg E, Becker N. Gene polymorphisms in Toll-like receptors, interleukin-10, and interleukin-10 receptor alpha and lymphoma risk. *Genes Immun*. 2006;7(8):615-624.
54. Flerlage JE, Myers JR, Maciaszek JL, et al. Discovery of novel predisposing coding and noncoding variants in familial Hodgkin lymphoma. *Blood*. 2023;141(11):1293-1307.
55. Cozen W, Timofeeva MN, Li D, et al. A meta-analysis of Hodgkin lymphoma reveals 19p13.3 TCF3 as a novel susceptibility locus. *Nat Commun*. 2014;5:3856-3865.
56. Mahat U, Terzioglu MK, Buhtoiarov I. CTLA4 haploinsufficiency as a predisposition to classical Hodgkin lymphoma. *Pediatr Hematol Oncol*. 2020;37(2):176-183.
57. Record J, Sendel A, Kritikou JS, et al. An intronic deletion in megakaryoblastic leukemia 1 is associated with hyperproliferation of B cells in triplets with Hodgkin lymphoma. *Haematologica*. 2020;105(5):1339-1350.
58. Ristolainen H, Kilpivaara O, Kamper P, et al. Identification of homozygous deletion in ACAN and other candidate variants in familial classical Hodgkin lymphoma by exome sequencing. *Br J Haematol*. 2015;170(3):428-431.
59. Rotunno M, McMaster ML, Boland J, et al. Whole exome sequencing in families at high risk for Hodgkin lymphoma: identification of a predisposing mutation in the KDR gene. *Haematologica*. 2016;101(7):853-860.
60. Srivastava A, Giangiobbe S, Kumar A, et al. Identification of familial Hodgkin lymphoma predisposing genes using whole genome sequencing. *Front Bioeng Biotechnol*. 2020;8:179-189.
61. Nijland M, Veenstra RN, Visser L, et al. HLA dependent immune escape mechanisms in B-cell lymphomas: implications for immune checkpoint inhibitor therapy? *Oncoimmunology*. 2017;6(4):e1295202-1295209.
62. Boot MV, Schaapveld M, Van den Broek EC, et al. Pathology review identifies frequent misdiagnoses in recurrent classic Hodgkin lymphoma in a nation-wide cohort: implications for clinical and epidemiological studies authors. *Haematologica*. 2023;108(5):1349-1358.
63. Meier J, Jensen JL, Dittus C, Coombs CC, Rubinstein S. Game of clones: diverse implications for clonal hematopoiesis in lymphoma and multiple myeloma. *Blood Rev*. 2022;56:100986-100996.
64. Tiacci E, Venanzi A, Ascani S, et al. High-risk clonal hematopoiesis as the origin of AITL and NPM1-mutated AML. *N Engl J Med*. 2018;379(10):981-984.
65. Warren JT, Link DC. Clonal hematopoiesis and risk for hematologic malignancy. *Blood*. 2020;136(14):1599-1605.