Annals of Diagnostic Pathology 46 (2020) 151533

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Detection of cryptic *CCND1* rearrangements in mantle cell lymphoma by next generation sequencing

Katarzyna Polonis[a,1], Matthew J. Schultz[a,1], Horatiu Olteanu[b], James B. Smadbeck[c], Sarah H. Johnson[c], George Vasmatzis[c], Xinjie Xu[a], Patricia T. Greipp[a], Rhett P. Ketterling[a,b], Nicole L. Hoppman[a], Linda B. Baughn[a], Jess F. Peterson[a,*]

[a] Division of Laboratory Genetics and Genomics, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States of America
[b] Division of Hematopathology, Mayo Clinic, Rochester, MN, United States of America
[c] Center for Individualized Medicine-Biomarker Discovery, Mayo Clinic, Rochester, MN, United States of America

ARTICLE INFO

Keywords:
CCND1
IGH
IGK
Mantle cell lymphoma (MCL)
Next generation sequencing (NGS)
Mate-pair sequencing (MPseq)

ABSTRACT

The accurate detection of recurrent genetic abnormalities for most hematologic neoplasms is critical for diagnosis, prognosis and/or treatment. Rearrangements involving *CCND1* are observed in a subset of mature B-cell neoplasms and can be reliably detected by fluorescence *in situ* hybridization (FISH) in most cases. However, cryptic and complex chromosomal rearrangements may pose a technical challenge for accurate diagnosis. Herein, we describe two patients with suspected mantle cell lymphoma that lacked obvious *CCND1* rearrangements by FISH studies. A next generation sequencing (NGS) based assay, mate-pair sequencing (MPseq), was utilized in each case to investigate potential cryptic *CCND1* rearrangements and revealed cryptic insertional events resulting in *CCND1/IGH* and *CCND1/IGK* rearrangements. These cases demonstrate that NGS-based assays, including MPseq, are a powerful approach to identify cryptic rearrangements of clinical importance that are not detected by current clinical genomics evaluation.

1. Introduction

Thorough characterization of mature B-cell neoplasms is critical for accurate diagnosis and optimal patient treatment. While morphologic and immunophenotypic evaluation is sufficient to diagnose many mature B-cell neoplasms, the demonstration of recurrent chromosomal abnormalities are required for other diagnoses (*e.g.*, high-grade B-cell lymphoma with *MYC*, *BCL2* and/or *BCL6* rearrangements) [1]. Mantle cell lymphoma (MCL) is a mature B-cell neoplasm associated with up-regulation of *CCND1* due to a t(11;14)(q13;q32) (*CCND1/IGH*) detected in nearly 95% of cases. The detection of *CCND1/IGH* is useful to support a MCL diagnosis in unclear mature B-cell neoplasms [2-4]. In addition to the classic t(11;14), variant translocations involving *CCND1* and *IGK* [t(2;11)] or *IGL* [t(11;22)] have also been reported in MCL [5-8]. In addition, *CCND2* and *CCND3* rearrangements may also be present in MCL cases which lack a *CCND1* rearrangement [9].

Fluorescence *in situ* hybridization (FISH) is the current gold standard assay utilized to identify recurrent cytogenetic alterations in mature B-cell neoplasms; however, this methodology may not detect complex or cryptic rearrangements of diagnostic or prognostic significance [10]. Herein, we present two cases of cryptic *CCND1* rearrangements that were not detected using *CCND1/IGH* dual-color dual-fusion (D-FISH) or *CCND1* break-apart probe (BAP) sets. Both cryptic *CCND1* rearrangements were readily detected using a next generation sequencing (NGS) methodology termed mate-pair sequencing (MPseq).

2. Case 1

A 69-year-old female presented with a marked leukocytosis (white blood cell count of 96.2 × 10^9/L, normal range: 3.4–9.6 × 10^9/L), mild thrombocytopenia (platelet count of 89 × 10^9/L, normal range: 157–371 × 10^9/L), moderate to severe macrocytic anemia (hemoglobin 5.4 g/dl, normal range: 11.6–15 g/dl; red blood cell count of 1.74 × 10^{12}/L, normal range: 3.92–5.13 × 10^{12}/L) and a mean corpuscular volume of 104.6 fL (normal range: 78.2–97.9 fL). Peripheral

* Corresponding author at: Division of Laboratory Genetics and Genomics, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States of America.
E-mail address: peterson.jess@mayo.edu (J.F. Peterson).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.anndiagpath.2020.151533

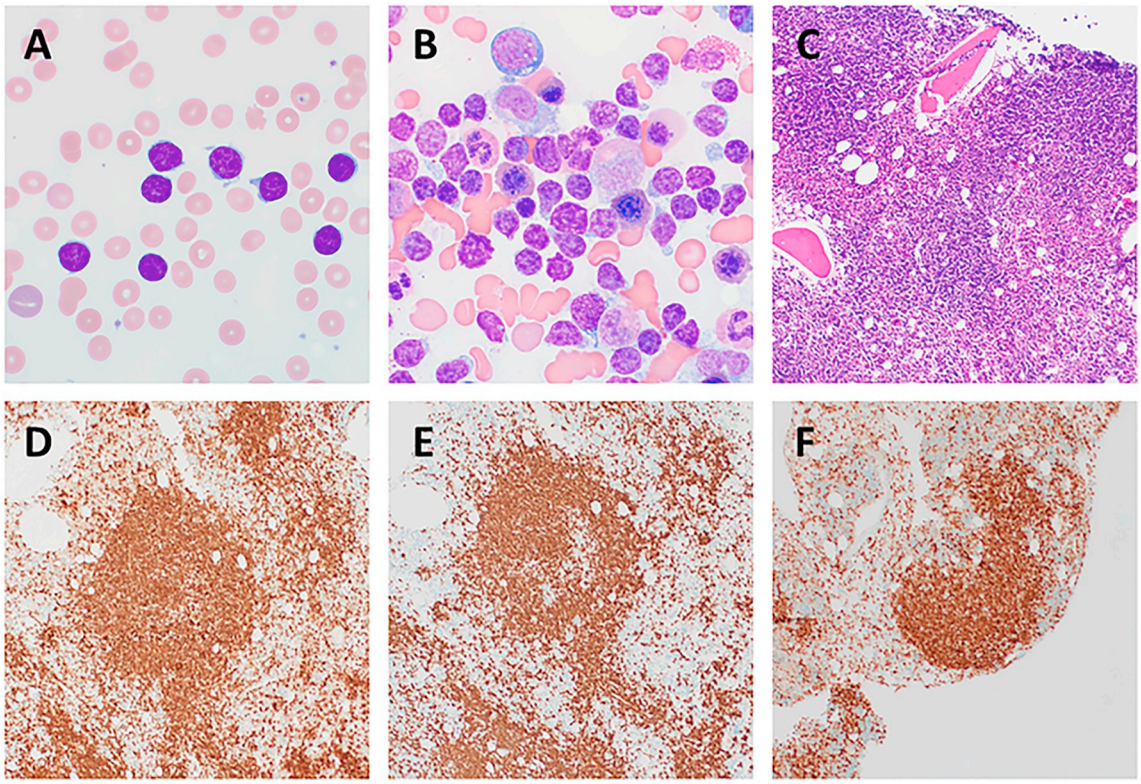

Fig. 1. Morphologic and immunophenotypic findings in case 1. (A) Medium-power (original magnification ×500) of a Wright-Giemsa stained peripheral blood smear showing atypical, small to medium-sized circulating lymphoma cells; (B) Wright-Giemsa stained bone marrow aspirate smear (original magnification ×500) shows an abnormal lymphocytic infiltrate with similar morphology to that described in the peripheral blood; (C) Hematoxylin and eosin–stained bone marrow biopsy (original magnification ×100) demonstrated a dense lymphoid infiltrate in an interstitial and nodular distribution. Immunohistochemical stains indicate that lymphoma cells are positive for CD20 (D), CD5 (E) and cyclin D1 (F).

blood smear examination indicated the marked lymphocytosis was comprised of atypical small to medium-sized cells, with round nuclear contours, condensed chromatin, and scant cytoplasm.

A bone marrow biopsy was performed and revealed a hypercellular bone marrow (90%) with 60% involvement by an abnormal lymphoid infiltrate, with nodular, focal paratrabecular, and interstitial distribution. Immunohistochemistry performed on the bone marrow core biopsy highlighted the nodular and interstitial infiltrates of CD20-positive B cells with co-expression of CD5 and cyclin D1. Trilineage hematopoiesis was slightly decreased and showed an erythroid predominance (myeloid to erythroid ratio = 1:1). Representative morphologic and immunophenotypic findings are illustrated in Fig. 1. Flow cytometry performed on the bone marrow aspirate demonstrated a kappa light-chain restricted B-cell population comprising approximately 80% of analyzed events, which was positive for CD19, CD20, CD22, CD5, FMC-7 and CD45 (bright), and negative for CD10, CD38, CD23, and CD200.

Conventional chromosome analysis from a CpG (B-cell mitogen) stimulated culture revealed the following complex karyotype: 44-45,X,-X,add(1)(q21),add(3)(p23), add(5)(q35),add(11)(p13),add(11)(q13),add(17)(p11.2),-22,del(22)(q11.2)[cp7]/44,sl,+8, -10,-add(11)(q13),+11,add(15)(p11.2)[2]/46,XX[1]. While the add(11)(q13) may suggest a potential *CCND1* alteration, concurrent *CCND1/IGH* D-FISH and *CCND1* BAP studies revealed no evidence of a *CCND1/IGH* rearrangement or disruption of the *CCND1* gene region, respectively (Fig. 2A,B). Despite the complexity of the clone by chromosome analysis, the only abnormality observed by FISH was a *TP53* gene deletion. Because of a strong suspicion of MCL, including positive cyclin D1 IHC staining, MPseq was subsequently performed and identified a "cryptic" insertional event involving ~86 kb of chromosome 11q13 inserted downstream of the *IGH* gene region on chromosome 14q32. This cryptic rearrangement results in insertion of the majority of *CCND1* into the *IGH* region, [hg38][ins(11;14)(14pter → 14q32.33(105,861,826)::11q13.3(69,565,131)-11q13.3(69,651,195)::14q32.33(106,153,574) → 14qter)] (Fig. 2C). At the gene level these breakpoints are predicted to result in truncation of the last exon in the *CCND1* transcript (NM_053056), suggesting the CCND1 transcript expressed would lack the 3' UTR.

3. Case 2

A bone marrow aspirate from an 83-year-old female with a suspected diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) was received for CLL FISH panel and flow cytometry studies. Flow cytometry demonstrated a B-cell clone with bright CD20, CD45, CD5 and surface kappa light chain expression, while negative for CD23, and negative to partial dim for CD200. These immunophenotypic features favored MCL, and would be unusual for CLL. No additional clinical information was available.

A D-FISH evaluation for t(11;14) indicated no *CCND1/IGH* rearrangement, but did reveal an additional diminished copy of the *CCND1* gene region in 86% of nuclei (Fig. 3A). The *CCND1* BAP assay demonstrated a normal signal pattern consistent with two intact *CCND1* gene regions despite the additional diminished *CCND1* signal observed by the *CCND1/IGH* D-FISH probe set (Fig. 3B). The extra diminished copy of *CCND1* raised suspicion for a cryptic *CCND1* rearrangement and MPseq was performed to further characterize this atypical FISH result. MPseq detected an insertional rearrangement involving chromosomes 2 and 11 that results in insertion of *CCND1* coding sequence into the *IGK* locus on chromosome 2, [hg38][(ins(2;11) (2pter → 2p11.2(88,859,563)::11q13.3(69,570,851) → 11q13.3(69,651,601)::2p11.2 (89,176,331) → 2qter)] (Fig. 3C). Similar to case 1, this rearrangement is predicted to result in a CCND1 transcript lacking the 3'UTR.

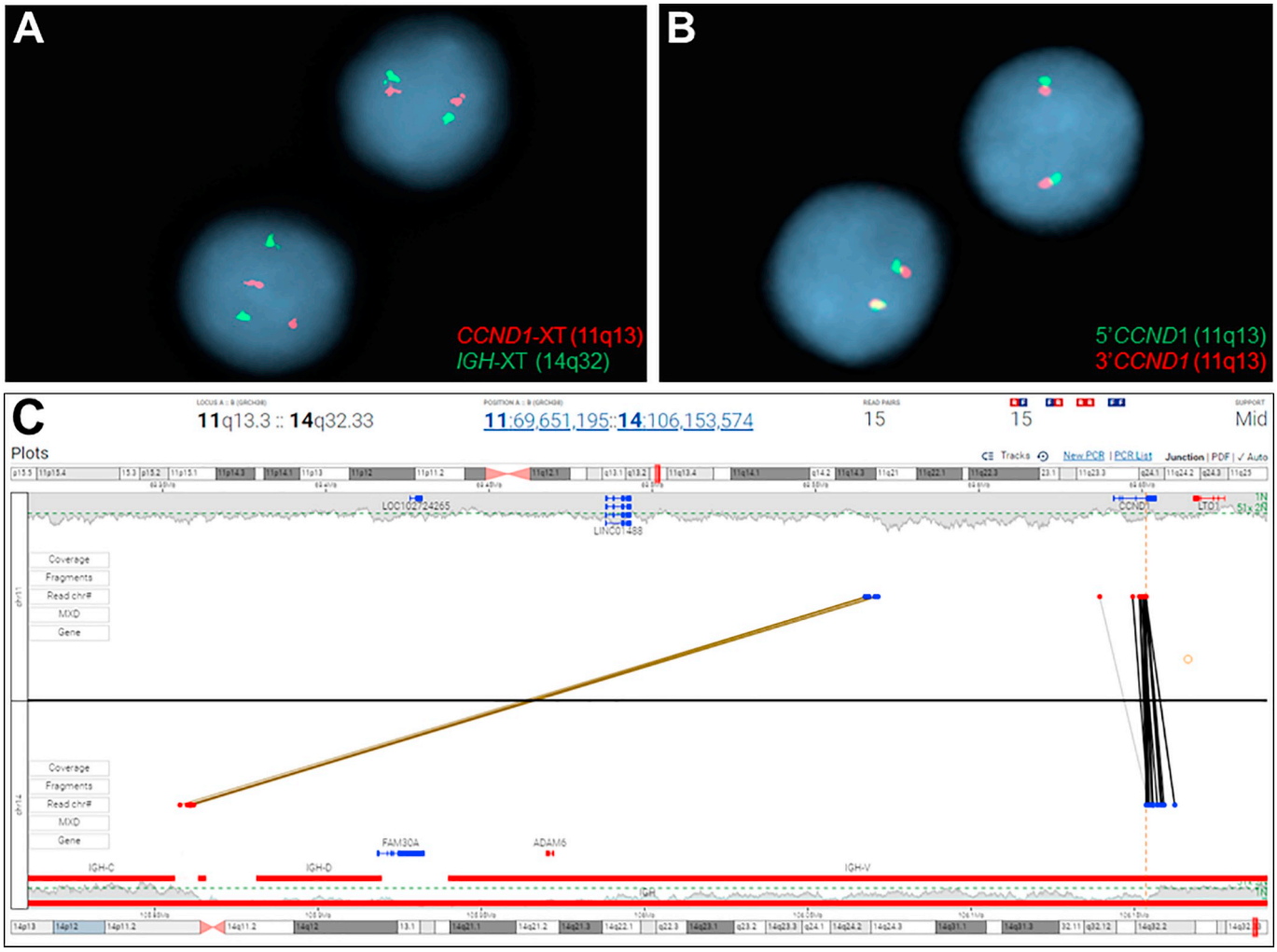

Fig. 2. Cytogenomic evaluation results in case 1. (A) Representative FISH image demonstrating a normal result of two red (*CCND1*) and two green (*IGH*) signals with *CCND1/IGH* dual-color dual-fusion probe set. (B) Normal result of two intact red/green fusion signals with *CCND1* break-apart probe set. (C) A junction plot from mate pair sequencing demonstrating an insertional rearrangement of the *CCD1* gene region into the *IGH* locus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Structural genomic rearrangements involving critical gene regions are increasingly important for diagnosing and managing hematologic malignancies [11]. While targeted FISH strategies detect many relevant genomic alterations, including *CCND1* rearrangements, cryptic or complex structural events may not be identified or fully characterized [5,12]. The two cases presented herein highlight the clinical utility of MPseq in characterizing *CCND1* cryptic rearrangements with greater precision than conventional cytogenetic approaches.

MPseq is an NGS-based technology that utilizes a library preparation and bioinformatics pipeline optimized to detect chromosomal structural variation. MPseq library preparation circularizes 2–5 kb DNA fragments followed by processing to short read size fragments which retain the spatial information of the longer read. Low pass genome-wide sequencing and secondary analysis utilizing a specialized aligner and caller designed for MP reads that can robustly detect structural variants and complex genomic rearrangements [13-15]. Unlike FISH probe strategies, MPseq is not limited to specific genomic footprints and provides an alternative technique to comprehensively detect and characterize structural rearrangements [16]. Precise identification and characterization of *CCND1* rearrangements is critical for the accurate diagnosis of MCL *versus* other mature B-cell lymphomas such as CLL/SLL [17].

In case 1, MPseq revealed a cryptic insertional translocation resulting in a *CCND1/IGH* rearrangement supporting the suspected MCL diagnosis after negative D-FISH and BAP FISH results (Table 1, Case 1). Although the *CCND1/IGH* rearrangement alone portends a more favorable prognosis in MCL, both the concurrent deletion of the *TP53* gene region and the overall chromosomal complexity suggests an unfavorable prognosis for this clone [18-22]. Loss of *TP53* combined with a *CCND1/IGH* rearrangement is often associated with the blastoid variant of MCL and predicts a poor response to chemoimmunotherapy treatment and indicates a more aggressive clinical course [23]. Similarly, we have previously published a single case report of cyclin D1-positive B-cell lymphoma with features overlapping with MCL where MPseq was also used to resolve a cryptic *CCND1/IGH* rearrangement (Table 1, Case 3) [12]. However, the only abnormality identified by FISH was an additional *IGH* signal, with a normal *CCND1* result for D-FISH and BAP FISH studies. MPseq detected an insertional rearrangement resulting in *CCND1/IGH* and ultimately established a diagnosis of MCL in this patient.

In case 2 with a suspected diagnosis of CLL, D-FISH studies demonstrated an additional diminished copy of *CCND1*, and the *CCND1* BAP study did not indicate a *CCND1* rearrangement. MPseq in this case confirmed a *CCND1* rearrangement identifying an insertion of the majority of the *CCND1* gene region into the *IGK* enhancer region (Table 1, Case 2). The immunophenotypic profile and molecular findings is more

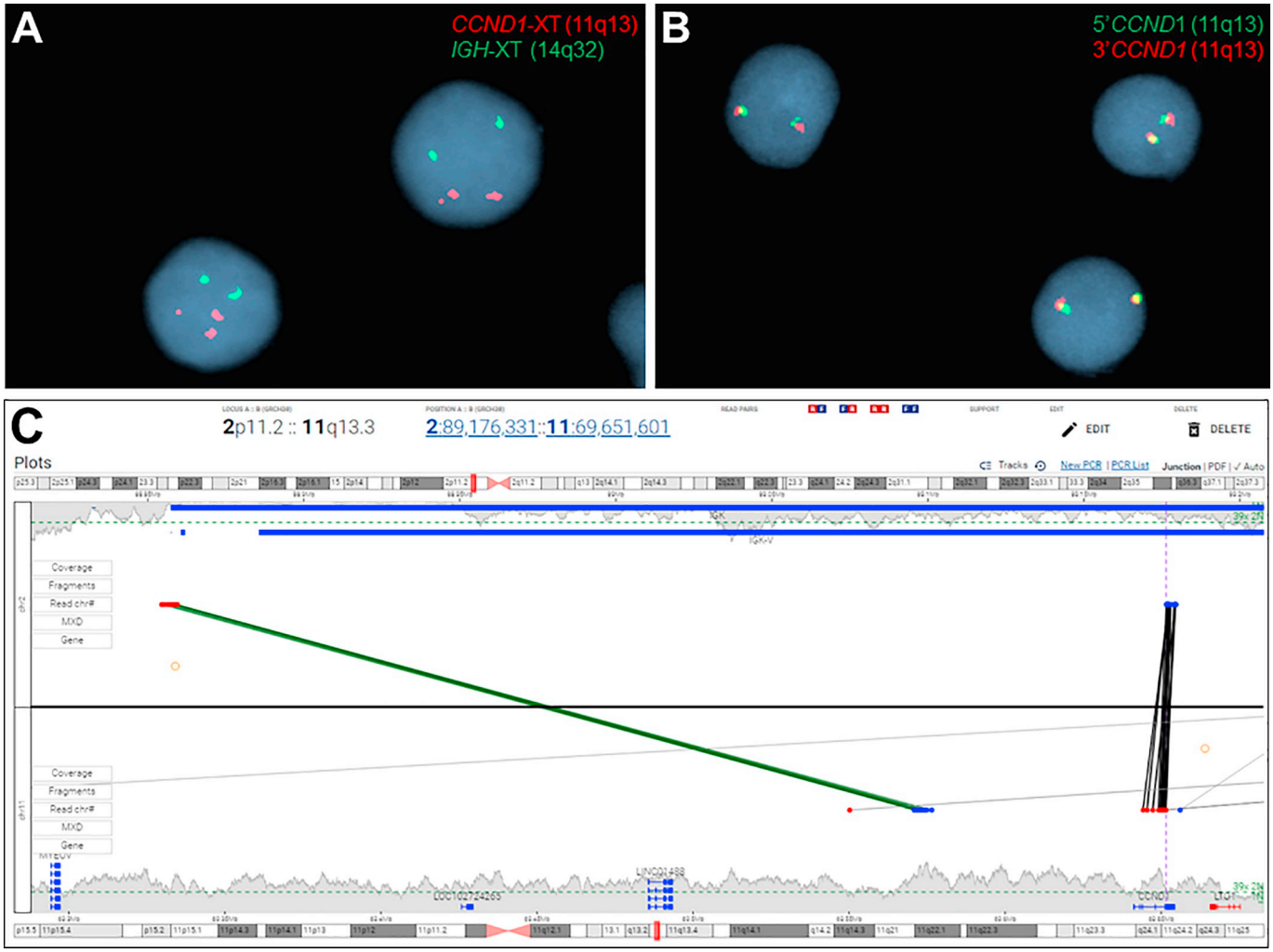

Fig. 3. Cytogenomic evaluation results in case 2. (A) Representative FISH images demonstrating an abnormal result of an extra (third) smaller *CCND1* signal and two normal *IGH* signals with *CCND1/IGH* dual-color dual-fusion probe set. No CCND1/IGH rearrangement was detected. (B) Normal result of two intact red/green fusion signals using a *CCND1* break-apart probe set. (C) A junction plot from mate pair sequencing demonstrating an insertion rearrangement of the *CCND1* gene region into the *IGK* locus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

consistent with a laboratory diagnosis of MCL, rather than a typical CLL. Rearrangements involving *CCND1* and *IGK* are rare and have been reported in less aggressive indolent B cell leukemia/lymphoma rather than classic MCL [5-7]. However, this patient's clone also exhibited a *TP53* deletion which combined with an *CCND1/IGK* rearrangement likely indicates a more aggressive neoplasm [19-22,24]. Fuster et al. recently reported a series of four MCL and one mature B-cell lymphoma with insertional translocations of *CCND1* into the *IGK* promoter region [6]. These cases were cyclin D1 positive by IHC and similarly lacked evidence of t(11;14). After the index case was analyzed by genome sequencing, custom FISH probes were developed to identify *CCND1/IGK* rearrangements. These cases highlight insertional translocations involving the *IGK* locus as an additional driver of *CCND1* expression which can be potentially missed or misinterpreted due to incomplete characterization by current diagnostic testing. In addition, we previously characterized a similar genomic alteration by MPseq in a plasma cell neoplasm that harbored the t(2;11) *CCND1/IGK* rearrangement (Table 1; case 4) [5]. The *CCND1/IGH* D-FISH studies revealed an additional *CCND1* probe signal, but with no apparent evidence of *CCND1/IGH* rearrangement. MPseq in this case identified a *CCND1/IGK* rearrangement. However, subsequent *CCND1* BAP studies did reveal a *CCND1* rearrangement.

For cases 1 and 2 described herein, the inserted *CCND1* gene lacked the 3′ UTR, suggesting a shortened CCND1 transcript. *CCND1* transcripts lacking the 3′ UTR have been shown to be more stable leading to increased cell proliferation and a worse prognosis than expression of full length *CCND1* [31]. The 3′ UTR is a site of negative regulation of Cyclin D1 by miR-16-1 and truncated transcripts escape this level of regulation contributing to increased proliferation [32]. NGS-based characterization of B-cell malignancies has the potential to characterize transcript level events capturing additional alterations which may have molecular and clinical relevance.

In conclusion, mounting evidence demonstrates that further genomic characterization by NGS methodologies complements conventional chromosomes and FISH testing for hematologic malignancies, especially for cryptic, atypical or complex rearrangements and to identify unknown partner genes [11,25-27,30]. The two cases presented herein add to the growing body of literature that NGS-based assays have a role in detecting cryptic genomic alterations and can serve to further characterize rearrangements that may impart transcript level alterations. Wider adoption and integration of NGS-based technologies should be considered in challenging patient cases to improve diagnostic accuracy which will ultimately benefit patients and their clinicians.

Table 1
Cryptic *CCND1* rearrangements detected by mate-pair sequencing in mature B-cell neoplasms.

	Case 1	Case 2	Case 3 (PMID: 31015206)	Case 4 (PMID: 31320253)
Sex	Female	Female	Female	Female
Age (years)	69	83	79	56
Diagnosis	MCL	Immunophenotypic findings most consistent with MCL	MCL	Plasma cell neoplasm
CCND1 FISH results	*CCND1/IGH* D-FISH: negative; *CCND1* BAP: negative	*CCND1/IGH D-FISH:* extra diminished *CCND1* signal; *CCND1* BAP: negative	*CCND1/IGH D-FISH:* extra *IGH* signal; *CCND1* BAP: negative	*CCND1/IGH D-FISH:* extra *CCND1 signal; CCND1* BAP: positive
MPseq results	Cryptic *CCND1/IGH* fusion	Cryptic *CCND1/IGK* fusion	Cryptic *CCND1/IGH* fusion	*CCND1/IGK* fusion

BAP, break-apart probe; D-FISH, dual-color dual-fusion; FISH, fluorescence *in situ* hybridization; MCL, mantle cell lymphoma; MPseq, mate-pair sequencing.

Declaration of competing interest

GV: Algorithms described in this manuscript for mate-pair sequencing are licensed to WholeGenome LLC owned by GV. The remaining authors have no conflicts of interest to disclose.

References

[1] Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. Blood 2018;131:2283–96.

[2] Li JY, Gaillard F, Moreau A, et al. Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. Am J Pathol 1999;154:1449–52.

[3] Bentz JS, Rowe LR, Anderson SR, et al. Rapid detection of the t(11;14) translocation in mantle cell lymphoma by interphase fluorescence in situ hybridization on archival cytopathologic material. Cancer 2004;102:124–31.

[4] Pérez-Galán P, Dreyling M, Wiestner A. Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era. Blood 2011;117:26–38.

[5] Peterson JF, Meyer RG, Smoley SA, et al. Whole genome mate-pair sequencing of plasma cell neoplasm as a novel diagnostic strategy: a case of unrecognized t(2;11) structural variation. Clin Lymphoma Myeloma Leuk 2019;19:598–602.

[6] Fuster C, Martín-Garcia D, Balagué O, et al. Cryptic insertions of the immunoglobulin light chain enhancer region near CCND1 in t(11;14)-negative mantle cell lymphoma. Haematologica 2019. https://doi.org/10.3324/haematol.2019.237073. [In press].

[7] Wlodarska I, Meeus P, Stul M, et al. Variant t(2;11)(p11;q13) associated with the IgK-CCND1 rearrangement is a recurrent translocation in leukemic small-cell B-non-Hodgkin lymphoma. Leukemia 2004;18:1705–10.

[8] Rocha CK, Praulich I, Gehrke I, et al. A rare case of t(11;22) in a mantle cell lymphoma like B-cell neoplasia resulting in a fusion of IGL and CCND1: case report. Mol Cytogenet 2011;4:8.

[9] Martín-Garcia D, Navarro A, Valdés-Mas R, et al. CCND2 and CCND3 hijack immunoglobulin light-chain enhancers in cyclin D1−mantle cell lymphoma. Blood 2019;133:940–51.

[10] Prakash G, Kaur A, Malhotra P, et al. Current role of genetics in hematologic malignancies. Indian J Hematol Blood Transfus 2016;32:18–31.

[11] Tran AN, Taylan F, Zachariadis V, et al. High-resolution detection of chromosomal rearrangements in leukemias through mate pair whole genome sequencing. PLoS ONE 2018;13:e0193928.

[12] Peterson JF, Baughn LB, Ketterling RP, et al. Characterization of a cryptic IGH/CCND1 rearrangement in a case of mantle cell lymphoma with negative CCND1 FISH studies. Blood Adv 2019;3:1298–302.

[13] Johnson SH, Smadbeck JB, Smoley SA, et al. SVAtools for junction detection of genome-wide chromosomal rearrangements by mate-pair sequencing (MPseq). Cancer Genet 2018;221:1–18.

[14] Smadbeck JB, Johnson SH, Smoley SA, et al. Copy number variant analysis using genome-wide mate-pair sequencing. Genes Chromosomes Cancer 2018;57:459–70.

[15] Drucker TM, Johnson SH, Murphy SJ, et al. BIMA V3: an aligner customized for mate pair library sequencing. Bioinformatics 2014;30:1627–9.

[16] Smadbeck J, Peterson JF, Pearce KE, et al. Mate pair sequencing outperforms fluorescence in situ hybridization in the genomic characterization of multiple myeloma. Blood Cancer J 2019;9:103.

[17] Jares P, Colomer D, Campo E. Molecular pathogenesis of mantle cell lymphoma. J Clin Invest 2012;122:3416–23.

[18] Yang P, Zhang W, Wang J, et al. Genomic landscape and prognostic analysis of mantle cell lymphoma. Cancer Gene Ther 2018;25:129–40.

[19] Woyach JA, Lozanski G, Ruppert AS, et al. Outcome of patients with relapsed or refractory chronic lymphocytic leukemia treated with flavopiridol: impact of genetic features. Leukemia 2012;26:1442–4.

[20] Haferlach C, Dicker F, Schnittger S, et al. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. Leukemia 2007;21:2442–51.

[21] Van Dyke DL, Werner L, Rassenti LZ, et al. The Dohner fluorescence in situ hybridization prognostic classification of chronic lymphocytic leukaemia (CLL): the CLL research consortium experience. Br J Haematol 2016;173:105–13.

[22] Parikh SA, Strati P, Tsang M, et al. Should IGHV status and FISH testing be performed in all CLL patients at diagnosis? A systematic review and meta-analysis. Blood 2016;127:1752–60.

[23] Espinet B, Salaverria I, Beà S, et al. Incidence and prognostic impact of secondary cytogenetic aberrations in a series of 145 patients with mantle cell lymphoma. Genes Chromosomes Cancer 2010;49:439–51.

[24] Bagacean C, Tempescul A, Ternant D, et al. 17p deletion strongly influences rituximab elimination in chronic lymphocytic leukemia. J Immunother Cancer 2019;7:22.

[25] Abel HJ, Al-Kateb H, Cottrell CE, et al. Detection of gene rearrangements in targeted clinical next-generation sequencing. J Mol Diagn 2014;16:405–17.

[26] Rowsey RA, Smoley SA, Williamson CM, et al. Characterization of TCF3 rearrangements in pediatric B-lymphoblastic leukemia/lymphoma by mate-pair sequencing (MPseq) identifies complex genomic rearrangements and a novel TCF3/TEF gene fusion. Blood Cancer J 2019;9:81.

[27] Peterson JF, Pitel BA, Smoley SA, et al. Use of mate-pair sequencing to characterize

a complex cryptic BCR/ABL1 rearrangement observed in a newly diagnosed case of chronic myeloid leukemia. Hum Pathol 2019;89:109–14.
[30] Aypar U, Smoley SA, Pitel BA, et al. Mate pair sequencing improves detection of genomic abnormalities in acute myeloid leukemia. Eur J Haematol 2019;102:87–96.
[31] Wiestner A, Tehrani M, Chiorazzi M, et al. Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival. Blood 2007;109:4599–606.
[32] Chen RW, Bemis LT, Amato CM, et al. Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma. Blood 2008;112:822–9.