

LONGITUDINAL MOLECULAR CHARACTERIZATION OF A RARE *TPM3::PDGFRB*-REARRANGED MYELOID/LYMPHOID NEOPLASM WITH LATE CNL-LIKE NEUTROPHILIC EVOLUTION

Frédéric Lambert¹, Benjamin Koopmansch¹, Rafael Fernandez Carazo¹, Elisa Dardenne¹, Aurore Keutgens², Gaëtan Vanstraelen³, Patrick Collins⁴, Catherine Menten¹, Gaëlle Vertenoel⁵

¹Center for Human Genetics, University Hospital of Liège, Liège, Belgium

²Department of Laboratory Medicine, Hematology Laboratory, University Hospital of Liège, Liège, Belgium

³Department of Pathology, University Hospital of Liège, Liège, Belgium

⁴Department of Hematology, Regional Hospital of East-Belgium, Verviers, Belgium

⁵Department of Hematology, University Hospital of Liège, Liège, Belgium

Corresponding Author

Frédéric Lambert (frederic.lambert@chuliege.be)

Keywords

Hypereosinophilia ; myeloid/lymphoid neoplasm with tyrosine kinase fusion ; *PDGFRB* ; *TPM3::PDGFRB*

ABSTRACT

Objectives: Myeloid/lymphoid neoplasms with tyrosine kinase fusions are rare but important because many are highly sensitive to targeted therapy. *PDGFRB* rearrangements are well-established drivers, whereas *TPM3* is an exceptionally rare fusion partner.

Methods: We performed longitudinal analysis of an elderly patient with hypereosinophilia, integrating morphology, cytogenetics, *PDGFRB* break-apart FISH, whole-transcriptome RNA sequencing, optical genome mapping, targeted myeloid DNA sequencing and exploratory fusion-specific RT-q PCR.

Results: A balanced t(1;5)(q21;q33) with *PDGFRB* rearrangement in 70% of nuclei was identified at diagnosis. RNA sequencing detected an in-frame *TPM3* exon 8-*PDGFRB* exon 11 fusion, and optical genome mapping confirmed the balanced rearrangement. Low-dose imatinib induced durable haematologic and cytogenetic remission of the fusion-driven eosinophilic component. Serial DNA sequencing identified diagnostic *ASXL1*, *EZH2* and low-level *SRSF2* abnormalities that persisted during remission, followed by acquisition or expansion of *SETBP1*, *CSF3R*, *ETNK1* and a distinct *SRSF2* variant during late neutrophilic progression, without re-emergence of the *PDGFRB* rearrangement.

Conclusions: *TPM3::PDGFRB* is a rare but actionable *PDGFRB* fusion. Durable suppression of the fusion-positive eosinophilic component was followed by a late CNL-like neutrophilic phase. The clonal relationship between these phases remains unresolved. Integrated longitudinal fusion testing and myeloid mutational profiling are therefore essential to characterize disease complexity.

Introduction

Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK) constitute a distinct clinicopathologic category defined by constitutive activation of a fusion kinase and remarkable sensitivity to tyrosine kinase inhibitors (TKIs). Rearrangements involving *PDGFRA*, *PDGFRB*, *FGFR1*, and more recently *JAK2*, represent the prototypical genetic drivers of these entities [1]. Among these, rearrangements of *PDGFRB*, located at 5q31–q33, are well-established and typically confer rapid and durable responses to imatinib, even at low doses [2–4]. In most *PDGFRB*-rearranged MLN-TK reported to date, the genomic profile is dominated by the driver fusion with few or low-burden additional myeloid mutations, and truly fusion-only cases are not uncommon, particularly in younger patients [3, 5].

Although numerous fusion partners of *PDGFRB* have been described, most are rare, and the clinical spectrum associated with individual partners remains incompletely characterized [6–9]. *TPM3* is an exceptionally uncommon partner in *PDGFRB*-rearranged MLN-TK, with only two cases reported to date in the indexed literature, predominantly in pediatric or young adult patients [6, 10, 11]. Detailed longitudinal molecular characterization of such cases is lacking, particularly in elderly individuals, in whom clonal hematopoiesis and age-related myeloid mutations may coexist [12].

Recent classifications have emphasized that kinase-driven myeloid neoplasms may coexist with, or arise within, a broader clonal haematopoietic context [5]. The concept of “associated haematologic neoplasm” (AHN) highlights that a highly TKI-sensitive fusion-driven component does not necessarily represent the entirety of the disease biology [1, 5]. Indeed, accumulating evidence indicates that additional somatic mutations—particularly in epigenetic regulators and spliceosome genes—may precede or accompany tyrosine kinase fusions, potentially influencing disease phenotype, therapeutic response, and long-term evolution [5, 13]. However, longitudinal genomic data documenting clonal dynamics before, during, and after TKI exposure in *PDGFRB*-rearranged MLN-TK remain scarce [3, 13].

Here, we describe an elderly patient with *TPM3::PDGFRB*-rearranged MLN-TK who achieved durable remission of the fusion-driven eosinophilic component under low-dose imatinib but subsequently developed a late CNL-like neutrophilic myeloid phase. Through integrated cytogenetic, transcriptomic, optical genome mapping, and serial DNA sequencing analyses, we document the longitudinal molecular evolution of this unusual case and discuss alternative clonal models, including linear evolution of the fusion-positive disease, branching evolution from an antecedent myeloid clone, and emergence of a distinct associated myeloid neoplasm.

Methods

PATIENT AND CLINICAL DATA

Clinical, laboratory, and follow-up data were retrospectively collected from medical records. Investigations were performed as part of routine diagnostic care in an ISO 15189-accredited laboratory and in accordance with institutional guidelines and the Declaration of Helsinki.

CYTOGENETICS AND FISH

Conventional bone marrow karyotyping used standard G-banding, with at least 20 metaphases analyzed at diagnosis. Interphase FISH used a commercial *PDGFRB* break-apart probe.

FUSION-SPECIFIC *TPM3::PDGFRB* RT-QPCR

Exploratory fusion-specific RT-qPCR for *TPM3::PDGFRB* was performed retrospectively on selected archived samples, using *ABL1* as reference transcript and the diagnostic peripheral blood sample as calibrator. Reverse transcription used 40 ng equivalent total RNA/ μ L in 20 μ L with the Ipsogen RT Kit on a Veriti thermocycler, according to the manufacturer's instructions.

Fusion-specific primers MRD-F and MRD-R described by Li et al. were used for *TPM3::PDGFRB* amplification [10]. *ABL1* served as reference transcript according to the Europe Against Cancer strategy [14]. Reactions were run in 25 μ L with LightCycler 480 SYBR Green I Master chemistry on a LightCycler 480 II instrument (Roche) under the following cycling conditions: 95°C for 5 min, then 45 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 30 s. Product specificity was assessed by melting-curve analysis. Relative transcript levels were calculated using the $2^{-\Delta\Delta C_t}$ method and expressed relative to the diagnostic baseline sample.

Given the retrospective nature of this assay and the limited availability of archived RNA, results were considered exploratory and were not used as a formal standardized MRD endpoint. An empirical interpretive threshold, corresponding to approximately 0.45% of the diagnostic baseline, was defined during retrospective analysis of the RT-qPCR follow-up data and displayed in Figure 4 only as a visual aid for longitudinal interpretation. This threshold was not used to define standardized molecular response or measurable residual disease categories.

TRANSCRIPTOME SEQUENCING AND FUSION ANALYSIS

Total RNA extracted from the diagnostic bone marrow sample was initially analyzed by untargeted poly(A)-selected whole-transcriptome RNA sequencing to identify the unknown *PDGFRB* fusion partner suggested by cytogenetics and *PDGFRB* break-apart FISH. Libraries were prepared according to validated laboratory protocols. Sequencing data were analyzed using the laboratory workflow available at testing, including alignment and manual inspection of fusion-supporting reads in Integrative Genomics Viewer. This analysis identified *TPM3* as the 5' fusion partner of *PDGFRB* and

detected exonic reads consistent with a *TPM3::PDGFRB* fusion transcript. Intronic mate-pair reads mapping to *TPM3* and *PDGFRB* guided targeted PCR and refined localization of the chromosomal breakpoints.

Exon and intron numbering for *TPM3* and *PDGFRB* throughout this report is based on the current MANE Select transcripts *TPM3* NM_152263.4 and *PDGFRB* NM_002609.4, unless otherwise specified.

TARGETED RNA SEQUENCING

The *TPM3::PDGFRB* fusion identified by whole-transcriptome RNA sequencing was subsequently confirmed on diagnostic RNA using the FusionPlex ALL panel (ArcherDX; AK0074 v1.6). Data were analyzed with Archer Analysis pipeline version 7.3.2 using the Fusion and SNP/InDel workflows, with inclusion of non-targeted mutations. Sample, variation and fusion quality controls passed predefined pipeline criteria. The run generated 963 677 reads after adapter trimming, with 99.9% mapped fragments and 99.2% on-target fragments. The reportable *TPM3::PDGFRB* fusion was classified as Tier I, supported by 2950 reads and 471 unique start sites, and joined *TPM3* exon 8 (NM_152263.4) to *PDGFRB* exon 11 (NM_002609.4).

OPTICAL GENOME MAPPING

Optical genome mapping was performed on ultra-high molecular weight DNA using the Saphyr system (Bionano Genomics, San Diego, CA, USA), according to the manufacturer's workflow and laboratory procedures. DNA molecules were DLE-1-labelled, counterstained, loaded onto nanochannel chips and imaged on the Saphyr instrument. Image files were converted into digital molecule maps and analyzed for structural variants and copy-number changes using the Rare Variant Pipeline in Bionano Solve version 3.7, with alignment to the human reference genome hg19/GRCh37. Data were reviewed using Bionano Access version 1.8.2. Large cytogenetically visible structural and copy-number variants greater than 5 Mb were reported, together with smaller variants involving genes or genomic regions of diagnostic or prognostic relevance in haematologic malignancies. Structural variants were interpreted in conjunction with conventional cytogenetics, FISH, and RNA sequencing, with particular attention to rearrangements involving chromosomes 1 and 5 and the *TPM3* and *PDGFRB* loci.

TARGETED DNA NEXT-GENERATION SEQUENCING

Targeted myeloid DNA sequencing was performed on diagnostic and follow-up samples using a validated 99-gene myeloid panel based on the Extended Myeloid Solution workflow (SOPHiA Genetics) and sequenced on an Illumina NextSeq instrument. Data were analyzed with SOPHiA DDM version 7.12.1.1 using the ILL1XG1S5_CNV_NextSeq_14 pipeline, with alignment to the human reference genome GRCh37/hg19. Variants were annotated using JAX Clinical Knowledgebase. The analytical limit of detection was approximately 4% variant allele fraction; values below 5% were reported as < 5%. Variants were interpreted according to pathogenicity, recurrence in myeloid neoplasms, variant allele fraction, sequencing depth and longitudinal behavior across serial samples. Variants with stable allele

frequencies close to 50% across time points were considered non-informative for longitudinal somatic clonal interpretation in the absence of paired germline testing.

Results

INITIAL PRESENTATION AND DIAGNOSIS

A 77-year-old man was referred in November 2014 for evaluation of marked hypereosinophilia. Peripheral blood examination showed leukocytosis ($11.1 \times 10^9/\text{L}$) with an absolute eosinophil count of $2.94 \times 10^9/\text{L}$ (26.4%), an absolute neutrophil count of $5.83 \times 10^9/\text{L}$ (52.4%), an absolute monocyte count of $0.69 \times 10^9/\text{L}$ (6.2%), and an absolute basophil count of $0.07 \times 10^9/\text{L}$ (0.6%), normal hemoglobin, and normal platelet count. Serum tryptase was within the laboratory reference range at $10.9 \mu\text{g}/\text{L}$. No evidence of allergic, infectious, autoimmune, or secondary causes of eosinophilia was identified. Clinical examination and imaging were unremarkable (Table 1).

Bone marrow examination revealed a hypercellular marrow with increased eosinophilic precursors and mature eosinophils, representing approximately 12% of nucleated cells, without blast increase ($< 5\%$). Reticulin staining showed mildly increased reticulin fibres, corresponding to MF-1. The available trephine biopsy report did not describe compact mast-cell aggregates or spindle-shaped mast-cell infiltrates. These findings supported a clonal eosinophilic myeloid neoplasm and prompted molecular work-up for eosinophilia-associated kinase rearrangements. *FIP1L1–PDGFRB* testing was negative (Figure 1A) [15, 16].

Conventional cytogenetics demonstrated a balanced $t(1;5)(q21;q33)$ in 20 of 21 metaphases examined, supporting a diagnosis of myeloid-lymphoid neoplasm with tyrosine kinase gene fusion and hypereosinophilia (MLN-TK) (Figure 2A). Interphase and metaphase FISH using a *PDGFRB* break-apart probe confirmed rearrangement in $\sim 70\%$ of nuclei, establishing a diagnosis of *PDGFRB*-rearranged MLN-TK (Figure 2B,C). At that time, commercial agnostic RNA-sequencing assays were not available in our routine diagnostic workflow. Untargeted poly(A)-selected whole-transcriptome RNA sequencing was performed to identify the unknown *PDGFRB* fusion partner suggested by cytogenetics and *PDGFRB* break-apart FISH. This analysis identified an in-frame *TPM3::PDGFRB* fusion joining *TPM3* exon 8 to *PDGFRB* exon 11. Under the WHO 2008 classification applicable in 2014, these findings established a myeloid/ lymphoid neoplasm with eosinophilia and *PDGFRB* rearrangement. The case would currently be classified as a *PDGFRB*-rearranged MLN-TK according to WHO-HAEM5 and ICC 2022.

Targeted RNA sequencing using an anchored multiplex PCR-based commercial panel and optical genome mapping were subsequently performed retrospectively on archived material. Targeted RNA sequencing confirmed the expressed *TPM3::PDGFRB* fusion using a routine-compatible assay, whereas optical genome mapping confirmed the balanced $t(1;5)(q21;q33)$ involving the *TPM3* and *PDGFRB* loci and did not identify additional clinically relevant structural or copy-number abnormalities. These retrospective analyses complemented the original diagnostic work-up but were not required to establish the initial actionable diagnosis.

Retrospective targeted myeloid DNA sequencing of the diagnostic sample revealed pathogenic *ASXL1* p.(Gln512*) and *EZH2* c.2195 + 1G>A variants, together with a low-level *SRSF2* p.(Pro95His) variant (Figure 3 and Table 2).

RESPONSE TO IMATINIB

Imatinib was initiated at 100 mg/day in December 2014. A rapid haematologic response was observed, with normalization of leukocyte counts and near-complete suppression of eosinophilia within days. Five months after treatment initiation, bone marrow evaluation showed marked reduction of eosinophils to 4.3%, persistent chronic-phase morphology and no blast increase. Disease monitoring under TKI therapy relied on haematologic assessment and *PDGFRB* FISH. Fusion-specific RT-qPCR was performed retrospectively on archived samples as an exploratory assay and was not used for real-time treatment monitoring. *PDGFRB* FISH was negative, consistent with complete haematologic and cytogenetic response (Figure 3 and Table 1).

Under continuous low-dose imatinib, the eosinophilic component remained durably controlled during 5 years before progression of the disease to the neutrophilic phenotype.

EMERGENCE OF A NEUTROPHILIC MYELOID PHENOTYPE

Although overt peripheral blood neutrophilic leukocytosis became apparent in 2019, retrospective review showed that marrow abnormalities were already detectable in September 2018. Bone marrow aspiration at that time showed hypercellular marrow with granulocytic predominance and maturation shifted toward mature neutrophils. Nucleocytoplasmic maturation asynchrony and hypogranular or agranular terminal granulocytic forms were reported, together with occasional small or monolobated megakaryocytes. Eosinophils remained quantitatively and morphologically within normal limits, and no blast excess was observed.

By September 2019, bone marrow aspiration showed markedly hypercellular marrow with granulocytic predominance and prominent maturation abnormalities, predominantly affecting granulation and involving more than 10% of granulocytic cells. The erythroid lineage was hypoplastic, whereas megakaryocytes were increased and included small dysplastic forms on aspirate smears. However, the corresponding trephine biopsy did not show a dominant population of atypical small hypolobated megakaryocytes typical of MDS/MPN with neutrophilia. No marrow eosinophilia or blast excess was identified. Interpretation of the degree of dysplasia was partly limited by a reported fixation or processing artifact. The corresponding trephine biopsy, although of limited representativeness, showed approximately 95% cellularity, granulocytic hyperplasia with maturation shifted toward segmented forms, relative erythroid hypoplasia, and mildly increased megakaryocytes with occasional abnormal nuclear hypersegmentation. Eosinophils were slightly increased but without frank excess. Immunohistochemical staining for CD34 and CD117 showed no blast excess, and reticulin fibrosis was absent (MF-0). The morphologic differential diagnosis at that stage included a reactive marrow response and an evolving myeloproliferative neoplasm.

TABLE 1 | Longitudinal clinicopathological, cytogenetic and molecular evolution of a *TPM3::PDGFRB*-rearranged myeloid/lymphoid neoplasm with late CNL-like neutrophilic evolution.

Parameters	Initial eosinophilic <i>PDGFRB</i> -rearranged MLN-TK	Early response under imatinib—2015–2016	Emerging neutrophilic phenotype under continued imatinib—2018–2019	Established late CNL-like neutrophilic phase—2019/2020
Treatment	Initially no treatment; imatinib 100 mg/day initiated on 8 Dec 2014	Imatinib 100 mg/day continued	Imatinib 100 mg/day continued	Imatinib discontinued (Dec 2019); hydroxyurea initiated
Peripheral blood counts	Hb 14.0 g/dL; WBC $11.2 \times 10^9/L$; Platelets $205 \times 10^9/L$	Hb 15.1–15.5 g/dL; WBC $5.3\text{--}7.2 \times 10^9/L$; Platelets $189\text{--}209 \times 10^9/L$	Hb 15.1–13.7 g/dL; WBC $14.92 \times 10^9/L$; Platelets $223\text{--}177 \times 10^9/L$	Hb 13.0–8.6 g/dL; WBC $42.1\text{--}64.6 \times 10^9/L$; Platelets $116\text{--}60 \times 10^9/L$
Monocytes	$0.69 \times 10^9/L$ (6.2%)	$0.19\text{--}0.40 \times 10^9/L$ (3.5%–5.6%)	$0.13 \times 10^9/L$ (0.9%); no monocytosis	$0.72\text{--}0.84 \times 10^9/L$ (1.3%); no monocytosis
Immature granulocytes/metamyelocytes	Not reported	Not increased	Immature granulocytes 3.5% ($0.52 \times 10^9/L$) in September 2019	December 2019: immature granulocytes 7.8% (< 10%), with myelocytes 3% and metamyelocytes 2%
Eosinophils	$2.94 \times 10^9/L$ (26.4%)	$0.13\text{--}0.15 \times 10^9/L$ (2.1%–2.6%)	$0.19\text{--}0.23 \times 10^9/L$ (1.5%–2.0%); no recurrent eosinophilia	November 2020: immature granulocytes 8.2% (< 10%); $0.49\text{--}0.06 \times 10^9/L$ (0.9%–0.1%); no recurrent eosinophilia
Bone marrow aspirate	Hypocellular marrow with eosinophilia (~12%); blasts < 5%	Marrow with eosinophils reduced to 4.3%; no blast excess	Hypocellular marrow with granulocytic predominance, right-shifted maturation and dysgranulopoietic changes; erythroid hypoplasia; no eosinophilia or blast excess	Markedly hypocellular marrow with granulocytic hyperplasia, right-shifted maturation, dysgranulopoiesis and erythroid hypoplasia; without recurrent eosinophilia or blast excess
Trephine biopsy (bone marrow)	Hypocellular marrow with eosinophilic infiltration; no compact mast-cell aggregates reported	Not available	~95% cellularity with hyperplasia and relative erythroid hypoplasia; without a dominant population of atypical small hypolobated megakaryocytes; MF-0	~95% cellularity with granulocytic hyperplasia; mildly dysplastic megakaryocytes without a dominant MDS/MPN-N pattern; MF-0/1
Blasts/flow cytometry	Blasts 2.99% by flow cytometry; no blast excess morphologically (< 5%)	No blast excess; flow cytometry not available	CD34/CD117-positive blasts 0.8%–1.7%; no blast progression	CD34/CD117-positive blasts 1.5%–1.7%; no blast progression

(Continues)

TABLE 1 | (Continued)

Parameters	Initial eosinophilic PDGFRB-rearranged MLN-TK	Early response under imatinib—2015–2016	Emerging neutrophilic phenotype under continued imatinib—2018–2019	Established late CNL-like neutrophilic phase—2019/2020
Cytogenetics/PDGFRB FISH	46, XY, t(1;5) (q21;q33) [20]; PDGFRB break-apart FISH positive (~70%)	Normal karyotype; PDGFRB break-apart FISH negative	No confirmed clonal cytogenetic evolution; PDGFRB break-apart FISH negative	No confirmed clonal cytogenetic evolution; PDGFRB break-apart FISH negative
Molecular findings	TPM3::PDGFRB; ASXL1; EZH2; low-level SRSF2	Persistent ASXL1 and EZH2; TPM3::PDGFRB transcript markedly reduced	Persistent ASXL1 and EZH2 variants; no re-emergence of the PDGFRB rearrangement	Persistent ASXL1 and EZH2; acquisition of CSF3R, SETBP1, ETNK1 and distinct SRSF2; TPM3::PDGFRB transcript remained detectable at very low level
Response	Not applicable	Complete hematologic and cytogenetic response	Response maintained	Progressive neutrophilic evolution despite persistent suppression of the TPM3::PDGFRB-positive component
Integrated interpretation	WHO 2008: CEL with PDGFRB rearrangement; WHO-HAEM5/ICC 2022: PDGFRB-rearranged MLN-TK	Durable suppression of the fusion-driven eosinophilic MLN-TK under low-dose imatinib	Emerging neutrophilic myeloid phenotype during sustained suppression of the TPM3::PDGFRB-positive component	Late CNL-like neutrophilic phase during sustained suppression of the TPM3::PDGFRB-positive component; MDS/MPN with neutrophilia remained in the differential diagnosis but was not favored

Note: The table summarizes the patient's clinical, haematologic, bone marrow, cytogenetic and molecular findings at diagnosis, during early response under imatinib, during emergence of the neutrophilic phenotype, and during the established late CNL-like neutrophilic phase.
Abbreviations: CNL, chronic neutrophilic leukemia; FISH, fluorescence in situ hybridization; Hb, hemoglobin; ICC, International Consensus Classification; MDS/MPN-N, myelodysplastic/myeloproliferative neoplasm with neutrophilia; MF, myelofibrosis grade; MLN-TK, myeloid/lymphoid neoplasm with tyrosine kinase fusion; WBC, white blood cell count; WHO, World Health Organization.

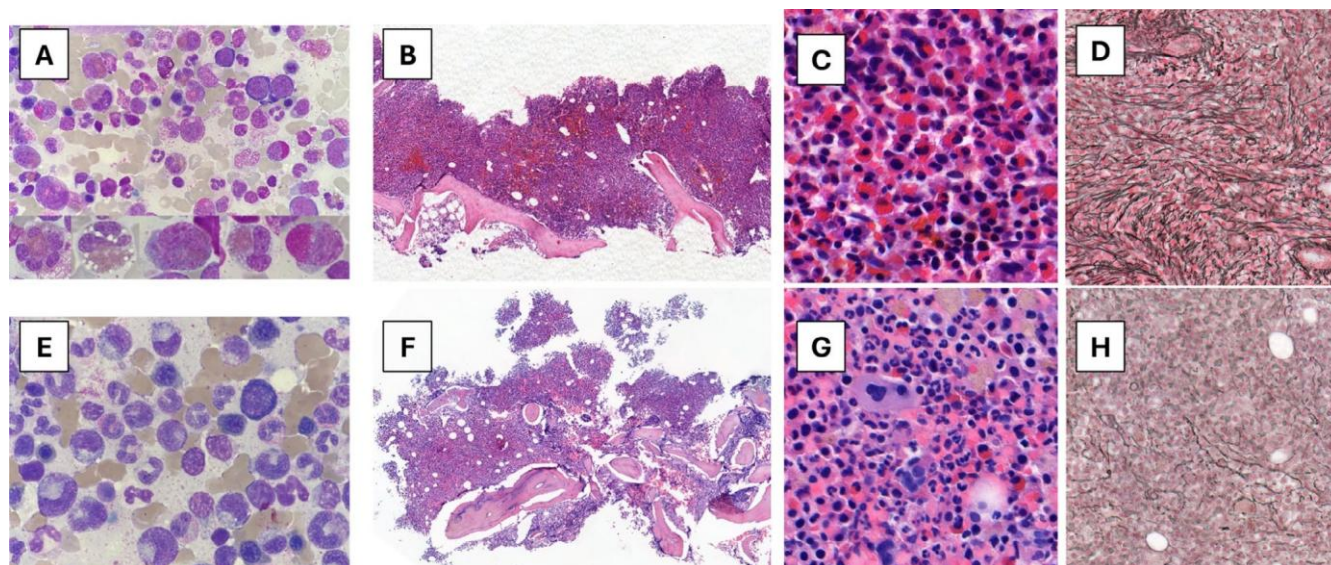


FIGURE 1 Bone marrow morphology at diagnosis, during imatinib-induced remission, and in the late CNL-like neutrophilic phase. (A–D) Diagnostic bone marrow findings: (A) aspirate smear (May–Grünwald–Giemsa, $\times 1000$), (B) low-power trephine biopsy overview (H&E), (C) higher-power H&E view, and (D) reticulin stain. (E) Bone marrow aspirate smear obtained during morphologic remission under low-dose imatinib. (F–H) Trephine biopsy obtained during the late neutrophilic phase, showing low-power H&E overview, higher-power H&E view, and reticulin stain. Overall, the figure illustrates the transition from an eosinophil-rich marrow at diagnosis to morphologic remission under imatinib, followed by a later neutrophil-predominant marrow phase corresponding to the late neutrophilic phase.

Conventional cytogenetic analysis of the September 2019 marrow did not provide evidence of clonal evolution. The karyotype was otherwise normal, and a single abnormal metaphase did not fulfill the criteria for a clonal cytogenetic abnormality. Interphase FISH analysis showed no evidence of a *PDGFRB* rearrangement (Table 1 and Figure 3).

From 2019 onward, progressive leukocytosis with marked neutrophilia and emerging cytopenias was documented, while eosinophil counts remained normal or only minimally increased (Table 1, Figures 1E–H and S1). Manual peripheral blood differentials performed on December 4 and 9, 2019, during an episode subsequently confirmed as *Campylobacter jejuni* infection, showed a modest left shift, with 2% metamyelocytes and 3% myelocytes on both occasions. The concomitant infectious and inflammatory context initially complicated the interpretation of the neutrophilic leukocytosis.

By December 23, 2019, the white blood cell count had increased to $55.24 \times 10^9/\text{L}$, driven by marked neutrophilia ($47.32 \times 10^9/\text{L}$; 85.7%). Automated differential counting showed 7.8% circulating immature granulocytes, corresponding to an absolute count of $4.32 \times 10^9/\text{L}$. The absolute eosinophil count was $0.49 \times 10^9/\text{L}$, without recurrent hypereosinophilia, while the monocyte count remained low in relative terms at $0.72 \times 10^9/\text{L}$ (1.3%). The platelet count was $163 \times 10^9/\text{L}$. C-reactive protein remained mildly elevated at 12.14 mg/L but had decreased from approximately 44–47 mg/L earlier that month. Serum tryptase, measured separately in mid-December, was within the laboratory reference range at 10.9 $\mu\text{g}/\text{L}$.

Bone marrow aspiration performed in February 2020 confirmed persistent marked hypercellularity for age, with granulocytic predominance, maturation through all stages, a shift toward mature neutrophils, and dysgranulation. The erythroid lineage was poorly represented, whereas

megakaryocytes were present in moderate numbers and showed platelet production. No increase in eosinophil precursors or blasts was observed.

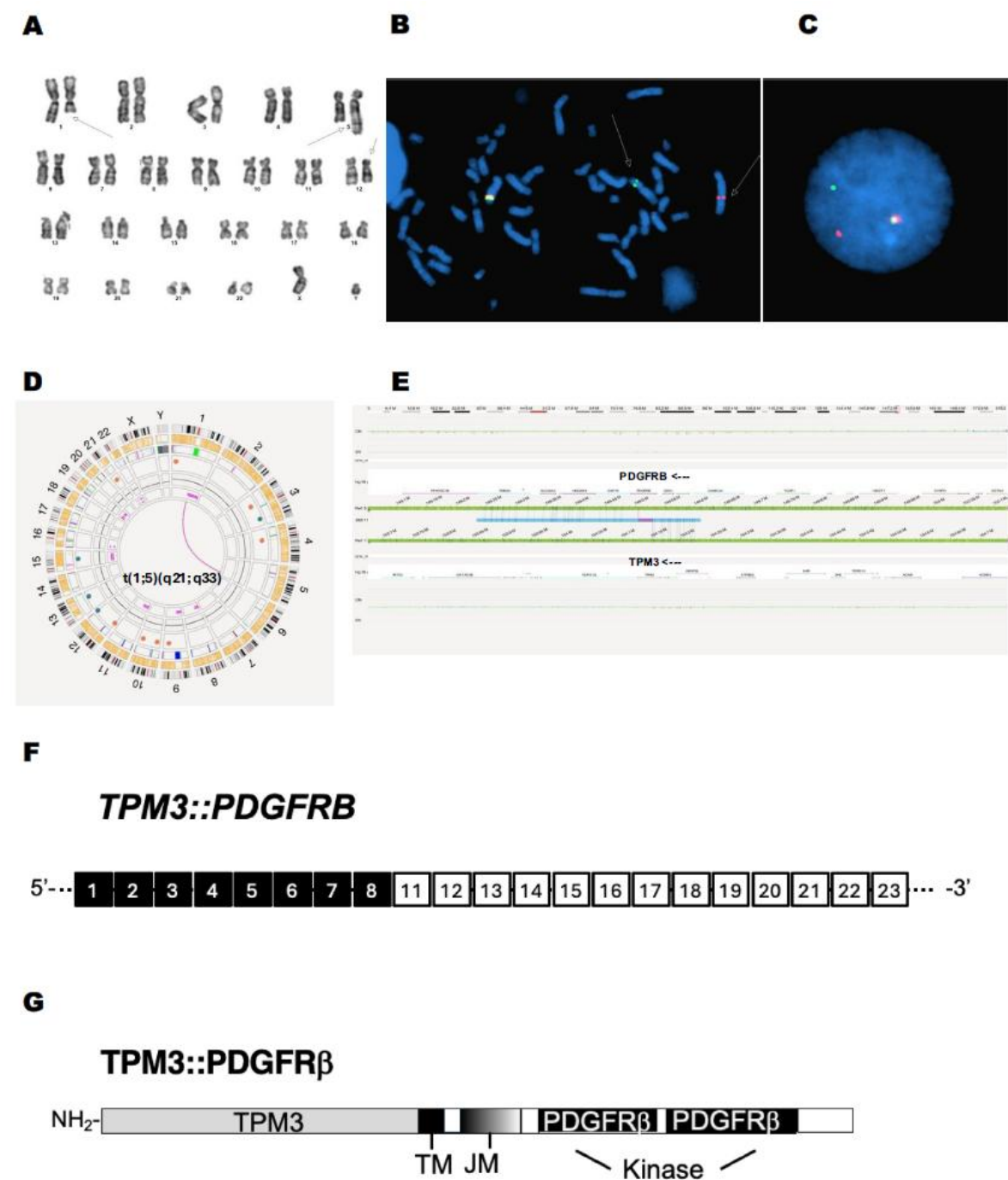


FIGURE 2 | Integrated structural characterization of the *TPM3::PDGFRB* fusion. (A) Conventional karyotype at diagnosis shows a balanced *t*(1;5)(q21;q33) in 20 of 21 analyzed metaphases, implicating the 5q33 *PDGFRB* locus. (B-C) Metaphase (B) and interphase (C) FISH with a *PDGFRB* break-apart probe demonstrate a split signal in 70% of nuclei at diagnosis, which becomes negative at 5 months under imatinib, consistent with cytogenetic remission of the MLN-TK clone. (D, E) Optical genome mapping confirming the *t*(1;5)(q21;q33)/*TPM3::PDGFRB* translocation. (D) Genome-wide circos plot showing a reciprocal translocation between chromosomes 1q21 and 5q32-33 (pink arc), annotated as a candidate *PDGFRB-TPM3* fusion. No major additional structural rearrangements are observed. (E) Linear view of the breakpoints at the *PDGFRB* locus on 5q32-33 (top) and the *TPM3* locus on 1q21 (bottom). Patient optical maps (green/blue) show molecules spanning the junctions between intronic regions of *TPM3* and *PDGFRB*, consistent with a balanced *t*(1;5)(q21;q33) generating the *TPM3::PDGFRB* fusion, without associated copy-number changes. (F) *TPM3::PDGFRB* fusion transcript with fusion between exon 8 of *TPM3* and exon 11 of *PDGFRB*. (G) Putative chimaeric protein with the retained coiled-coil motif of *TPM3* and the *PDGFRβ* tyrosine kinase domain.

T0 – Diagnosis Eosinophilic MLN-TK	T1 – On imatinib CHR / CCyR	T2 – Late CNL-like Neutrophilic phase
Cytogenetics / FISH		
46,XY,t(1;5)(q21;q33)[20]/46,XY[1]; PDGFRB break-apart FISH positive (70%)	46,XY[10]; PDGFRB break-apart FISH negative (0%)	46,XY [10+10c]; PDGFRB break-apart FISH negative (0%)
Fusion status		
TPM3::PDGFRB detected (RNA-seq; OGM-confirmed)	No detectable PDGFRB rearrangement by FISH	No evidence of TPM3::PDGFRB re-expansion
Bulk DNA NGS (VAF, %)		
ASXL1 p.(Gln512*) (45%) EZH2 c.2195+1G>A (36%) SRSF2 p.(Pro95His) (5%)	ASXL1 p.(Gln512*) (47%) EZH2 c.2195+1G>A (10%) SRSF2 p.(Pro95His) (31%)	ASXL1 p.(Gln512*) (47%) EZH2 c.2195+1G>A (48%) SRSF2 p.(Pro95Leu) (40%) SETBP1 p.(Asp868Asn) (47%) CSF3R p.(Thr618Ile) (26%) ETNK1 p.(Asn244Ser) (8%) TET2 p.(Cys1298Tyr) (42%)
Descriptive schematic (bulk samples); not a phylogenetic reconstruction or clonal hierarchy		

FIGURE 3 | Overview of longitudinal clinical and molecular findings in TPM3::PDGFRB-rearranged MLN-TK. Molecular analyses were performed on bulk samples and included RNA-based fusion testing and targeted DNA next-generation sequencing. The figure summarizes molecular findings documented at diagnosis, during sustained response under imatinib, and at late follow-up, alongside corresponding clinical and cytogenetic features. This schematic representation is based on bulk-sample data and is descriptive only; it is not intended to depict clonal hierarchy, phylogenetic relationships, or the temporal order of mutation acquisition. Bulk DNA NGS findings shown in the figure are restricted to pathogenic (P) or likely pathogenic (L/P) variants detected above the validated analytical limit of detection and considered clinically relevant to the longitudinal interpretation. T0, diagnosis of TPM3::PDGFRB-rearranged MLN-TK; T1, imatinib-induced haematologic and cytogenetic remission; T2, late CNL-like neutrophilic phase with acquisition of additional myeloid mutations; CHR, complete haematologic response; CCyR, complete cytogenetic response.

During 2020, anemia and thrombocytopenia progressively worsened, ultimately leading to transfusion dependence. An October 2020 trephine biopsy showed approximately 95% cellularity, marked granulocytic hyperplasia with immature forms in a peritrabecular distribution, and granulocytic dysplastic changes. Megakaryocytes were slightly increased, with hypolobated or monolobated forms reported, but these did not constitute a dominant morphologic pattern typical of MDS/ MPN with neutrophilia. The erythroid lineage was reduced to rare residual islands with occasional megaloblastoid forms. Immunohistochemical staining for MPO confirmed marked granulocytic hyperplasia, whereas CD34 and CD117 showed no blast excess. Reticulin staining demonstrated mild network densification (MF-1), without overt myelofibrosis.

On November 25, 2020, the last available complete blood count showed hemoglobin of 8.6 g/dL, a white blood cell count of $64.55 \times 10^9/L$, an absolute neutrophil count of $56.51 \times 10^9/L$, and 8.2% circulating immature granulocytes, corresponding to an absolute count of $5.31 \times 10^9/L$. The absolute eosinophil and monocyte counts were 0.06 and $0.84 \times 10^9/L$, respectively, with monocytes accounting for only 1.3% of leukocytes, and the platelet count was $60 \times 10^9/L$.

Across serial assessments from September 2018 to September 2020, bone marrow flow cytometry consistently showed only a small CD34/CD117 double-positive blast population, ranging from 0.8% to 1.7% without evidence of blast progression.

The patient died in June 2021 in the context of cardiac decompensation and infectious complications (Table 1).

TABLE 2 | Driver mutations and variant allele fractions across disease phases.

Genotyping results				Diagnosis	Haematologic and cytogenetic remission	Late CNL-like neutrophilic phase
Gene/Exon	c. HGVS	p. HGVS	Biological class	VAF (%)		
<i>ASXL1</i> : Exon12	c.1534C>T	p.(Gln512*)	4	45%	47%	47%
<i>EZH2</i> : Exon19	c.2195 + 1G>A	p.?	4	36%	10%	48%
<i>SRSF2</i> : Exon1	c.284C>T	p.(Pro95Leu)	5	< 5%	< 5%	40%
<i>SRSF2</i> : Exon1	c.284C>A	p.(Pro95His)	5	5%	31%	ND
<i>TET2</i> : Exon7	c.3893G>A	p.(Cys1298Tyr)	4	< 5%	< 5%	42%
<i>SETBP1</i> : Exon4	c.2602G>A	p.(Asp868Asn)	5	ND	ND	47%
<i>CSF3R</i> : Exon14	c.1853C>T	p.(Thr618Ile)	5	ND	ND	26%
<i>ETNK1</i> : Exon3	c.731A>G	p.(Asn244Ser)	4	ND	ND	8%
<i>TET2</i> : Exon10	c.4320dup	p.(Ser1441Glufs*37)	4	ND	< 5%	ND

Note: Values below the validated analytical reporting threshold are shown for longitudinal information only. *ASXL1* and *EZH2* persisted throughout follow-up, whereas *CSF3R*, *SETBP1*, *ETNK1*, and *SRSF2* p.(Pro95Leu) were acquired or markedly expanded during the late neutrophilic phase. Two distinct *SRSF2* variants were detected longitudinally: p.(Pro95His) at diagnosis and during remission, and p.(Pro95Leu) during the late neutrophilic phase. Variants with stable VAFs close to 50% across time points (*ATM* c.2770C>T; *FANCA* c.344G>A; and *FLT3* c.1691A>C) were considered non-informative for longitudinal somatic clonal interpretation in the absence of paired germline testing. Variant allele fractions are shown for pathogenic or clinically relevant variants detected at diagnosis (T0), during imatinib-induced haematologic and cytogenetic response (T1), and at late neutrophilic progression (T2). Variants with stable allele frequencies close to 50% across time points were considered non-informative for longitudinal clonal interpretation. Abbreviations: ND, not detected; VAF, variant allele fraction.

LONGITUDINAL MOLECULAR EVOLUTION

Serial molecular analyses were retrospectively performed using the samples collected at diagnosis (T0), during complete remission of the fusion-driven component under imatinib treatment (T1), and at neutrophilic progression (T2) (Figure 3 and Table S1).

At T1, *TPM3::PDGFRB* was no longer detectable by FISH, whereas *ASXL1* and *EZH2* persisted despite suppression of the fusion-positive component, supporting the presence of a sizeable antecedent myeloid clone not fully captured by fusion status alone. *SRSF2* remained involved across disease phases, although not through the same variant at T2 (Figure 3).

At T2, additional pathogenic variants characteristic of neutrophilic myeloid neoplasms emerged, including *SETBP1* p.(Asp868Asn), *CSF3R* p.(Thr618Ile), and *ETNK1* p.(Asn244Ser), without reappearance of the *PDGFRB* rearrangement (Figure 3 and Table 2).

A somatic *TET2* p.(Cys1298Tyr) variant was also detected at T2, increasing from a low variant allele fraction at T1 (< 5%) to 42% at T2. Variants with stable allele frequency near 50% were considered non-informative for longitudinal clonal interpretation (Figure 3).

These longitudinal bulk molecular findings documented persistence of the initial myeloid variants during remission of the fusion-driven component and the later emergence of additional variants during the neutrophilic phase.

Exploratory *TPM3::PDGFRB* RT-qPCR, normalized to *ABL1* and using the diagnostic peripheral blood sample as calibrator (set at 100%), showed similarly high fusion transcript levels in the first bone marrow sample collected before any TKI exposure (~95% of baseline), indicating a broadly disseminated *TPM3::PDGFRB*-positive clone in both peripheral blood and marrow at diagnosis. A subsequent sample obtained shortly after imatinib initiation still displayed fusion levels comparable to or slightly higher than baseline (~120%), consistent with the absence of an early log-scale molecular response. In contrast, later bone marrow samples collected under imatinib showed a marked reduction in *TPM3::PDGFRB* transcript levels, reaching approximately 0.03%, 0.01%, and 0.007% of baseline at 6, 12, and 24 months, respectively. Additional archived bone marrow samples obtained at 45.4, 57.1, and 62.4 months after TKI initiation, including during the late neutrophilic phase, remained at very low levels (0.0014%, 0.0005%, and 0.0007% of baseline, respectively), supporting sustained molecular suppression of the *TPM3::PDGFRB*-positive MLN-TK component throughout late follow-up (Figure 4).

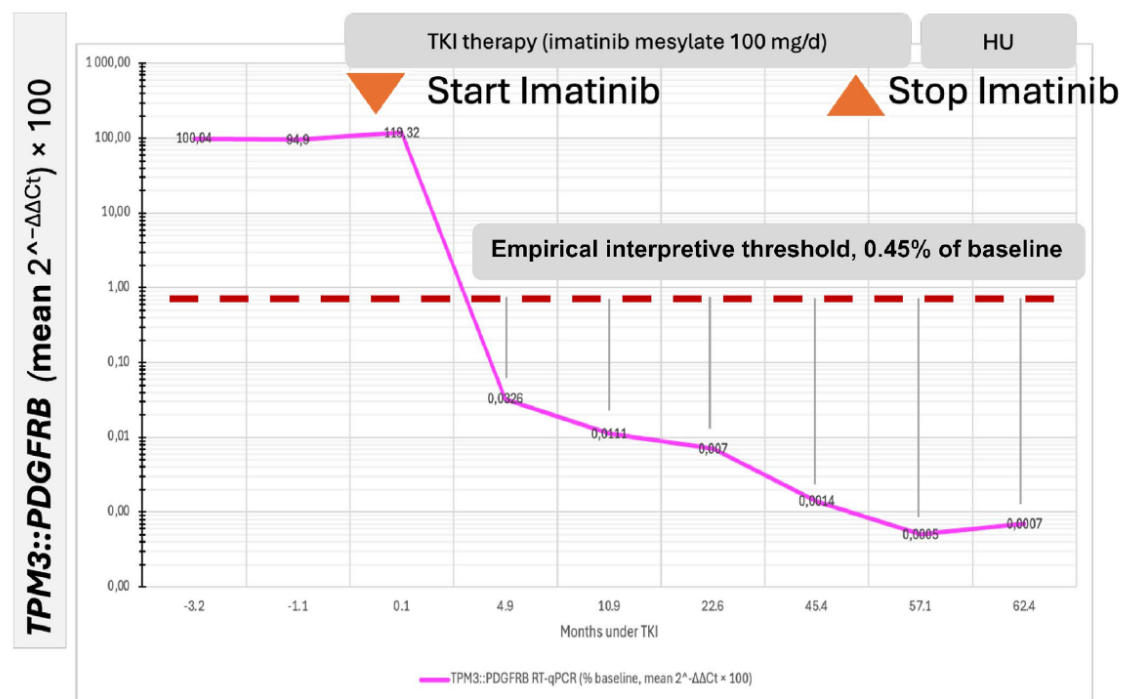


FIGURE 4 | Exploratory *TPM3::PDGFRB* RT-qPCR follow-up under imatinib. Exploratory fusion-specific *TPM3::PDGFRB* RT-qPCR follow-up performed retrospectively on available archived samples. Transcript levels were normalized to *ABL1* and expressed relative to the diagnostic peripheral blood sample, set at 100%. The graph shows high *TPM3::PDGFRB* transcript levels at diagnosis and shortly after imatinib initiation, followed by a marked decrease under low-dose imatinib and sustained very low levels during late follow-up, including during the late neutrophilic phase. The dashed line labelled “Empirical interpretive threshold” corresponds to an exploratory threshold defined during retrospective analysis and used only as a visual aid for longitudinal interpretation. It should not be interpreted as a validated analytical limit of quantification or as a standardized measurable residual disease threshold. RT-qPCR, reverse-transcription quantitative polymerase chain reaction; TKI, tyrosine kinase inhibitor.

Discussion

TPM3 is a rare but recurrent fusion partner of *PDGFRB* in MLN-TK. Although only two cases of *TPM3::PDGFRB*-rearranged MLN-TK have been reported in the literature, its repeated identification across independent patients supports biological selection rather than a purely stochastic event [6, 10,

11]. *TPM3* encodes a tropomyosin protein containing an N-terminal coiled-coil domain, which may provide a dimerization scaffold for ligand-independent activation of the retained *PDGFRβ* kinase domain in the chimeric protein. This mechanism is consistent with observations from other *TPM3*-associated kinase fusions, in which coiled-coil-mediated oligomerization contributes to constitutive kinase activation [17–20]. Experimental models of *TPM3::ALK* have demonstrated that progressive truncation of the coiled-coil domain reduces dimer formation and transforming capacity, directly linking this structural motif to oncogenic signaling [19, 20]. These observations provide a mechanistic framework for understanding the transforming potential of *TPM3::PDGFRB*.

Compared with other recurrent *PDGFRB* partners—such as *ETV6*, *BCR*, or *HIP1*—*TPM3* belongs to a class of cytoskeletal proteins whose intrinsic oligomerization domains function as strong dimerization scaffolds [17–20]. This structural configuration may influence signaling properties or subcellular localization, although its impact on lineage phenotype remains uncertain. The recurrence of *TPM3* across distinct kinase fusions suggests that its contribution extends beyond passive promoter swapping and reflects active structural selection during oncogenesis [18, 19, 21].

The rarity of *TPM3::PDGFRB* should not obscure its diagnostic importance [1, 15, 22–25]. Reported cases span heterogeneous clinical phenotypes, including CMML-like presentations, MDS/MPN overlap syndromes, and classical MLN-TK with or without eosinophilia, in both pediatric and adult patients [6–9, 26]. This diversity increases the risk of under-recognition, particularly when diagnostic workflows rely only on targeted assays or on panels with restricted fusion coverage.

In our patient's diagnostic journey, the clinical interpretation of the neutrophilic phase was initially challenging. Manual peripheral blood differentials performed on December 4 and 9, 2019 documented a modest left shift, with 2% metamyelocytes and 3% myelocytes on both occasions. Subsequent automated differentials showed increasing circulating immature granulocytes, from 4.0% on December 12 to 6.5% on December 16 and 7.8% on December 23. Interpretation was initially confounded by concomitant *Campylobacter jejuni* infection and systemic inflammation. At that time, reactive neutrophilia could not be reliably distinguished from recurrence of the fusion-driven neoplasm or emergence of a separate myeloid disorder. However, the leukocyte, neutrophil, and immature granulocyte counts continued to increase as C-reactive protein declined by December 23. Persistent neutrophilia and immature granulocytosis were subsequently documented throughout 2020, including 8.2% immature granulocytes, corresponding to an absolute count of $5.31 \times 10^9/L$, in November 2020, together with progressive anemia and thrombocytopenia. These findings supported a persistent neutrophilic myeloid phenotype rather than a purely infection-related leukemoid reaction, although the available peripheral blood differentials did not document $\geq 10\%$ circulating immature myeloid cells. Retrospectively, however, the persistence and constant progression of neutrophilia, cytopenias, transfusion dependence, and persistent marrow abnormalities during 2020 supported an underlying clonal process. Furthermore, the increased marrow cellularity already observed in September 2018, compared with the 2016 specimen, suggests that this evolution may have begun before the documented infectious episode.

In this setting, RNA-based fusion detection and cytogenomic approaches such as optical genome mapping provide complementary information: RNA sequencing identifies the expressed fusion transcript and partner gene, whereas optical genome mapping refines the structural architecture of

the rearrangement. Unbiased transcriptomic approaches may enhance sensitivity for detecting uncommon but actionable kinase rearrangements [27–32].

Available reports consistently indicate marked sensitivity of *PDGFRB*-rearranged neoplasms to imatinib, including cases harboring *TPM3* fusions [2, 33–36]. However, our case illustrates that clinical evolution may not be exclusively fusion-driven. Longitudinal mutational profiling provides additional insight into the clonal complexity of this case. The *ASXL1* p.(Gln512*) variant remained remarkably stable, with VAFs of approximately 45%–47% throughout the disease course. Although VAF alone cannot establish clonal hierarchy, this persistent high allelic burden is consistent with a dominant antecedent myeloid clone rather than a small incidental clonal haematopoietic population. Moreover, the *EZH2* splice-site variant c.2195 + 1G>A was already detected in the diagnostic sample at a VAF of 36%, indicating that molecular complexity preceded the development of the later neutrophilic phenotype. Its subsequent variation in VAF may reflect changes in clonal representation, treatment-related selection, or differences in sample composition.

By contrast, *CSF3R*, *SETBP1*, and *ETNK1* variants were detected above the validated analytical threshold only during the late neutrophilic phase. A distinct *SRSF2* p.(Pro95Leu) variant, previously observed only below the validated reporting threshold, expanded to 40% at this stage, whereas the *SRSF2* p.(Pro95His) variant identified at diagnosis and during remission was no longer detected. These findings are consistent with clonal diversification, although they do not establish whether the late neutrophilic phase arose through linear evolution, branching evolution from a shared antecedent clone, or emergence of a genetically distinct associated myeloid neoplasm.

However, the available bulk sequencing data do not establish whether the *TPM3::PDGFRB* fusion and the different somatic mutations occurred within the same cells, in linearly related subclones, or in genetically distinct branches. Consequently, several models remain plausible: linear evolution of the fusion-positive neoplasm, branching evolution from a shared ancestral myeloid clone, or the emergence of a distinct second myeloid neoplasm after control of the initial MLN-TK. Definitive discrimination between these possibilities would require clone-resolved approaches, such as single-cell DNA and/or RNA sequencing, colony-based genotyping, or molecular analysis of sorted haematopoietic cell populations.

Although *TPM3::PDGFRB* RT-qPCR was performed retrospectively and outside a standardized MRD framework, the assay documented high fusion transcript levels around diagnosis, followed by a greater than 4-log decrease in later bone marrow samples collected under imatinib. Importantly, transcript levels remained extremely low in archived samples obtained 45–62 months after TKI initiation, including during the late neutrophilic phase (Figure 4 and Table S1). Together with *PDGFRB* FISH negativity, these findings support deep and durable molecular suppression of the *TPM3::PDGFRB*-driven MLN-TK component.

The subsequent emergence of a neutrophilic phenotype despite sustained suppression of the *PDGFRB* rearrangement underscores the importance of comprehensive genomic profiling beyond fusion detection alone [5]. This interpretation is further supported by the mutational profile (Table 2). Unlike most *PDGFRB*-rearranged MLN-TK series, in which additional myeloid mutations are infrequent or of low variant allele fraction, our patient already harbored high-burden *ASXL1* and *EZH2* mutations at diagnosis, together with a low-level *SRSF2* variant. These findings may have therapeutic implications.

While TKI therapy effectively controls the kinase-driven component, identification of high-risk molecular features may influence long-term management decisions, including consideration of allogeneic haematopoietic stem cell transplantation in selected patients [5, 37]. Moreover, detection of actionable co-mutations such as *CSF3R* p.Thr618Ile may open alternative kinase-targeted strategies [38, 39]. Emerging agents targeting aberrant RNA splicing further illustrate how co-mutational architecture may become therapeutically relevant in the future [40].

Our observation also supports combined fusion testing and myeloid DNA profiling at diagnosis in suspected MLN-TK, particularly in older patients or in cases with cytopenias, dysplasia, neutrophilic evolution, or other features suggesting an additional myeloid substrate.

In summary, *TPM3::PDGFRB* represents a rare but biologically meaningful recurrent fusion whose detection has immediate therapeutic consequences. In this case, durable suppression of the fusion-positive eosinophilic component was followed by a late CNL-like neutrophilic phase, while the clonal relationship between these disease phases remained unresolved.

Our longitudinal molecular analysis highlights the necessity of integrating fusion status with comprehensive mutational profiling to accurately classify and manage *PDGFRB*-rearranged myeloid neoplasms.

AUTHOR CONTRIBUTIONS

Frédéric Lambert designed the study, performed molecular analyses, interpreted the data, and drafted the manuscript. Gaëlle Vertenoil provided critical revision of the manuscript. All authors contributed to the diagnosis and/or clinical management of the patient and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Andrée Villers, Miguel Lopez y Cadenas, Rudy Carpeaux, and Hélène Charlier for their excellent technical assistance. We are also grateful to Dr. Joan Somja, Nicolas Catarin, and Céline Lette for their scientific involvement in this case, and to Jean Bungert for providing clinical and biological data. The authors are grateful to the above-mentioned foundations for their early support of molecular diagnostic developments. During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to support language editing, readability improvement, manuscript formatting consistency, and editorial polishing. The authors reviewed and edited all AI-assisted outputs and take full responsibility for the final content of the manuscript.

FUNDING

This work was supported by research awards received by Frédéric Lambert in 2004 through the Léon Fredericq Foundation, including the Braconnier-Lamarque Foundation Prize, the Lempereur Foundation Prize, and the FIRS (Fonds d'Investigation pour la Recherche Scientifique), administered by the University Hospital of Liège.

ETHICS STATEMENT

This retrospective study was conducted using clinical data and residual diagnostic material obtained as part of routine care. No additional samples were collected for research purposes. According to institutional policy, patients are informed that residual biological material may be used for research unless they express opposition; the patient did not opt out. The study protocol has been submitted to the institutional ethics committee (reference number [CE 2026–111]). All procedures were conducted in accordance with the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All relevant clinical, cytogenetic, and molecular data supporting the findings of this study are included in the article and its [Supporting Information](#). Additional de-identified data are available from the corresponding author upon reasonable request, subject to institutional and ethical regulations governing patient data protection.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Longitudinal blood count profile under imatinib. **Table S1:** Exploratory longitudinal *TPM3::PDGFRB* RT-qPCR follow-up under imatinib.