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Case Report

Acute Myeloid Leukemia Following Mantle Cell Lymphoma: Clonal Evolution Versus Therapy-Related Pathogenesis—A Case Report with Longitudinal Molecular Tracking

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Abstract

We report a female in her late 80s with high-risk pleomorphic mantle cell lymphoma (MCL) harboring germline *ATM* mutation and 17p deletion who achieved complete metabolic response with zanubrutinib plus rituximab. Serial peripheral blood next-generation sequencing (NGS) during remission revealed emergence of a molecularly distinct pre-leukemic clone characterized by *DNMT3A*, *TET2*, and a *TP53* point mutation (p.Tyr220Cys)—distinct from the original 17p deletion. Approximately 20 months after MCL diagnosis, she developed acute myeloid leukemia (AML) with *FLT3*-TKD and *NPM1* mutations, confirming transformation of the pre-leukemic clone. Longitudinal VAF tracking demonstrated complete eradication of MCL-associated mutations (*ID3*, *BIRC3*) while the myeloid clone expanded. This case provides direct molecular evidence that AML arose from clonal evolution of a pre-existing hematopoietic clone rather than direct MCL transformation, with implications for understanding second malignancies in BTK inhibitor-treated patients.

Keywords: mantle cell lymphoma; acute myeloid leukemia; zanubrutinib; *TP53* mutation; clonal evolution; therapy-related myeloid neoplasm

1. Introduction

Mantle cell lymphoma is an aggressive B-cell non-Hodgkin lymphoma characterized by the t(11;14)(q13;q32) translocation resulting in cyclin D1 overexpression. *TP53* mutations, present in 20–30% of cases, represent the single most important molecular predictor of poor outcome, conferring primary refractoriness to conventional therapies and dramatically shortened survival. BTK inhibitors have revolutionized MCL treatment, with zanubrutinib demonstrating high efficacy and favorable tolerability as a highly selective second-generation agent.

While second primary malignancies occur in 9–22% of patients receiving BTK inhibitors, the specific risk of secondary myeloid neoplasms varies by agent [1]. MDS/AML has been reported with ibrutinib in the SHINE trial and is listed in the ibrutinib prescribing information, yet no cases of secondary myeloid neoplasms have been reported with zanubrutinib in clinical trials or post-marketing surveillance [2,3]. Distinguishing

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therapy-related malignancies from clonal evolution of pre-existing hematopoietic clones has significant implications for understanding disease pathogenesis and treatment selection.

Recent evidence demonstrates that hematopoietic stem cells from B-cell non-Hodgkin lymphoma patients, including MCL, may harbor premalignant clonal hematopoiesis mutations that can serve as the cellular origin of lymphoma. Wiegand et al. identified identical clonal hematopoiesis clones in 41% of corresponding lymphomas, with *TP53*-mutant clones showing tumor-promoting expansion, while single-cell genotyping confirmed mutated progenitors in MCL patients—providing direct evidence of a pre-neoplastic state in disease pathogenesis [4].

We present a case with comprehensive longitudinal molecular tracking demonstrating the emergence and expansion of a pre-leukemic clone during MCL remission, culminating in AML transformation.

2. Case Presentation

A female patient in her late 80s presented in 2023 with severe symptomatic anemia (hemoglobin 5.5 g/dL). Gastrointestinal bleeding and hemolysis were systematically excluded. Staging revealed infradiaphragmatic nodal disease, splenomegaly, and extranodal involvement. Two bone marrow biopsies were attempted: the first resulted in a dry tap suggestive of marrow infiltration, and the second was complicated by bleeding.

Lymph node biopsy confirmed pleomorphic MCL: positive for CD5, CD19, CD20, CD38, BCL-2, and CD43; negative for CD10, CD23, CD11c, and CD200; lambda light chain restriction; and FISH positive for *IGH::CCND1* translocation. Next-generation sequencing (NGS) revealed an ultra-high-risk molecular profile including presumed germline mutations in *ATM* (p.Lys468GlufsTer18, VAF 77%), *ID3* (p.Gln63Arg, VAF 50%), *BIRC3* (p.Asn130ThrfsTer17, VAF 46%), and *KMT2C* (p.Val961Ile, VAF 12%); chromosomal abnormalities including biallelic 17p deletion *(TP53 locus)*, small 11p deletion (*ATM locus*) with the concurrent aforementioned somatic frameshift *ATM* mutation, *CDKN2A/2B* deletion; and *BCL2* amplification (18q+) with markedly increased *BCL2*, *CCND1*, *SOX11* mRNA and complex karyotype. A possible germline *DPYD* variant (c.1236G>A, VAF 21%) was also identified. Immunohistochemistry demonstrated a Ki-67 proliferation index of 25%, and serum LDH was elevated at 476 U/L. Together, these findings established a diagnosis of high-risk pleomorphic MCL.

NGS on Peripheral Blood at diagnosis also revealed partially concordant but distinct findings: somatic mutations in *ID3* (p.Gln63Arg, VAF 43%), *BIRC3* (p.Asn130ThrfsTer17, VAF 42%), *KMT2C* (p.Val961Ile, VAF 8%), *NTRK3* (p.Glu590Lys, VAF 3%, predicted deleterious), and *SMC1A* (p.Arg816Ser, VAF 2%, predicted deleterious); presumed germline *ATM* mutation (p.Lys468GlufsTer18, VAF 69%); monoallelic 17p deletion (*TP53*); *CDKN2A/2B* deletion (9p-); possible germline *DPYD* mutation; and additional chromosomal abnormalities (1p-, 8p-, 15q-, 21q-). Notably, myeloid-associated genes including *DNMT3A*, *TET2*, *NPM1*, and *FLT3* were interrogated at this timepoint and were negative, indicating that the pre-leukemic myeloid clone was either absent or present below the assay's validated 1% VAF detection threshold.

She was treated with zanubrutinib 160 mg twice daily continuously plus rituximab. Peripheral blood flow cytometry performed 3 months after treatment initiation confirmed complete eradication of the MCL immunophenotype with virtual B-cell aplasia (CD19 0%, CD20 0%, CD22 0%; CD19+CD5+ 0%). The T-cell compartment demonstrated an inverted CD4:CD8 ratio (25% vs. 62%; ratio 0.4), with preserved total T cells (CD3 85%) and low-normal NK cells (CD56 10%). Quantitative immunoglobulin levels were not monitored. Serial PET-CT assessments demonstrated excellent metabolic response: Deauville 3 at 3 months, Deauville 2 at 9 months, and Deauville 1 at 14 months. Despite achieving

complete metabolic remission, peripheral blood MRD monitoring via NGS done during remission revealed a dramatically altered molecular landscape (Table 1). The original MCL-associated mutations (*ID3*, *BIRC3*) had decreased to near-undetectable levels (VAF 0.09% and 0.08%, respectively), and the 17p deletion was no longer identified. Strikingly, a new *TP53* point mutation (p.Tyr220Cys, VAF 0.59%) was detected, distinct from the original 17p deletion, alongside an emerging clone characterized by mutations in *DNMT3A* (p.Ala149Val, VAF 8.6%), *KMT2C* (p.Val961Ile, VAF 8.0%), *CTNNB1* (p.Ser646Cys, VAF 7.4%), *PIK3CA* (p.Gly364Glu, VAF 5.5%), *ZRSR2* (p.Asn261Ser, VAF 3.8%), *TET2* (p.Arg544Ter, VAF 1.5%), *BCORL1* (p.Pro340Leu, VAF 0.9%), *BCOR* (p.Pro904Leu, VAF 0.8%), and *MPL* (p.Pro2His, VAF 0.7%). This mutational profile, featuring *DNMT3A*, *TET2*, *TP53*, and splicing factor mutations, is characteristic of clonal hematopoiesis or a pre-leukemic myeloid clone.

Table 1. Longitudinal Molecular Profiling Across Disease Stages.

Gene	Variant	MCL Diagnosis (Tissue NGS)	MCL Diagnosis (PB NGS)	MCL Remission (PB NGS)	AML Diagnosis (PB NGS)	Interpretation
ATM	11q deletion	Detected	Not detected	Not detected	Not detected	MCL-specific chromosomal loss
ATM	p.Lys468GlufsTer18	VAF 77.0%	VAF 69.23%	Not detected	Not detected	Likely germline (high VAF); lost with MCL clone
TP53	17p deletion	Detected (biallelic)	Detected (monoallelic)	Not detected	Not detected	MCL-specific chromosomal loss
TP53	p.Tyr220Cys	Not detected	Not detected	VAF 0.59%	VAF 0.45%	AML-specific; pre-leukemic clone
ID3	p.Gln63Arg	Not detected	VAF 42.62%	VAF 0.09%	Not detected	MCL clone marker
BIRC3	p.Asn130ThrfsTer17	Not detected	VAF 42.09%	VAF 0.08%	Not detected	MCL clone marker
KMT2C	p.Val961Ile	VAF 12.05%	VAF 7.92%	VAF 7.98%	VAF 7.38%	Shared ancestral clone
NTRK3	p.Glu590Lys	Not detected	VAF 2.87%	Not detected	Not detected	MCL subclone
SMC1A	p.Arg816Ser	Not detected	VAF 2.17%	Not detected	Not detected	MCL subclone
DNMT3A	p.Ala149Val	Not detected	Not detected	VAF 8.6%	VAF 8.84%	CH/AML clone
DNMT3A	p.Gly10Arg	Not detected	Not detected	Not detected	VAF 0.8%	AML-specific
TET2	p.Arg544Ter	Not detected	Not detected	VAF 1.45%	VAF 0.03%	CH clone
CTNNB1	p.Ser646Cys	Not detected	Not detected	VAF 7.41%	VAF 6.07%	CH/AML clone
PIK3CA	p.Gly364Glu	Not detected	Not detected	VAF 5.49%	VAF 5.56%	CH/AML clone
ZRSR2	p.Asn261Ser	Not detected	Not detected	VAF 3.76%	VAF 3.11%	CH/AML clone
BCORL1	p.Pro340Leu	Not detected	Not detected	VAF 0.92%	VAF 0.78%	CH/AML clone
BCOR	p.Pro904Leu	Not detected	Not detected	VAF 0.75%	VAF 0.9%	CH/AML clone
MPL	p.Pro2His	Not detected	Not detected	VAF 0.66%	VAF 0.26%	CH/AML clone
FLT3	p.Asp839Gly (TKD)	Not detected	Not detected	Not detected	VAF 0.87%	AML-specific driver
GATA3	p.Phe153Val	Not detected	Not detected	Not detected	VAF 6.22%	AML-specific
DPYD	c.1236G>A (synonymous)	VAF 20.57%	VAF 22.66%	VAF 37.65%	VAF 37.74%	Likely germline

Abbreviations: MCL, mantle cell lymphoma; AML, acute myeloid leukemia; PB, peripheral blood; NGS, next-generation sequencing; VAF, variant allele frequency; CH, clonal hematopoiesis.

Twenty months after MCL diagnosis, the patient developed AML. Bone Marrow NGS at diagnosis confirmed expansion of the pre-leukemic clone with acquisition of

new mutations (Table 1). The *FLT3-TKD* mutation (p.Asp839Gly, VAF 0.87%) and a second *DNMT3A* mutation (p.Gly10Arg, VAF 0.8%) were newly detected. A novel *GATA3* mutation (p.Phe153Val, VAF 6.2%) was also identified. The previously detected mutations persisted: *DNMT3A* p.Ala149Val (VAF 8.8%), *KMT2C* (VAF 7.4%), *CTNNB1* (VAF 6.1%), *PIK3CA* (VAF 5.6%), *ZRSR2* (VAF 3.1%), *BCOR* (VAF 0.9%), *BCORL1* (VAF 0.8%), *TP53* p.Tyr220Cys (VAF 0.45%), *MPL* (VAF 0.26%), and *TET2* (VAF 0.03%). No chromosomal structural abnormalities were detected. Flow cytometry showed low-level blast marker expression (CD34, CD117, TdT) and low-level B-cell markers. Notably, a low-level T-cell clone (*TRGV4*) was detected, while B-cell clonality was absent, confirming eradication of the MCL clone. The original MCL-associated *ID3* and *BIRC3* mutations were no longer detectable (Figure 1).

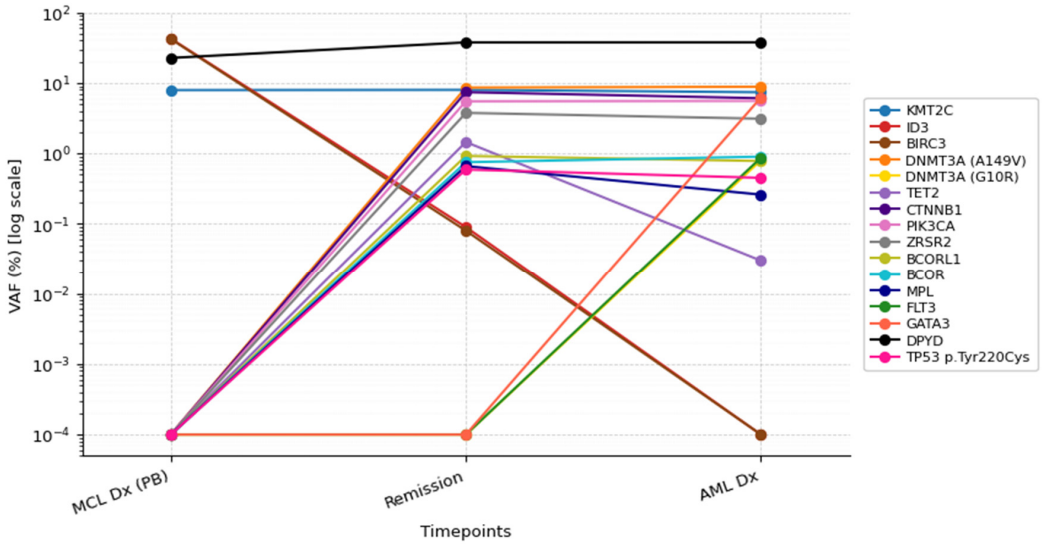


Figure 1. Longitudinal variant allele frequency (VAF) tracking demonstrating divergent clonal dynamics across disease stages.

All next-generation sequencing (NGS) testing was performed by Key Genomics/Anthology diagnostics using a comprehensive 284-gene hematologic malignancy panel with massive parallel sequencing of all coding exons and approximately 50 nucleotides of flanking intronic sequence. The panel encompasses genes associated with both lymphoid, and myeloid neoplasms, including *DNMT3A*, *TET2*, *NPM1*, *FLT3*, *IDH1*, *IDH2*, *RUNX1*, *TP53*, and *ASXL1*, among others. Fragment length analysis was additionally performed for *CALR*, *FLT3*, and *NPM1* to enhance detection of large indels. The validated analytical sensitivity is 1% variant allele frequency (VAF) for single-nucleotide variants (SNVs) and insertions-deletions (indels), and 2–5% for *CALR*, *FLT3-ITD*, and *NPM1* fragment analysis. Targeted RNA NGS analysis covering 1600 genes associated with hematologic neoplasms was performed concurrently using hybrid capture methodology, with a fusion detection sensitivity of 1–5%. The same comprehensive panel was used across all four timepoints (tissue NGS at MCL diagnosis, peripheral blood NGS at MCL diagnosis, peripheral blood NGS during remission, and bone marrow NGS at AML diagnosis). Critically, myeloid-associated genes were interrogated at all timepoints, including at MCL diagnosis. Variants reported below the 1% validated threshold were included in the analysis but should be interpreted with caution, as they fall below the assay's validated limit of detection.

3. Discussion

This case provides granular longitudinal molecular evidence supporting clonal evolution, rather than therapy-related mutagenesis, as the mechanism underlying AML development in a patient with high-risk MCL treated with zanubrutinib–rituximab. The molecular

data conclusively demonstrate that the AML arose from a pre-existing hematopoietic clone that was molecularly distinct from the MCL, and that this clone most likely predated zanubrutinib exposure. While the role of BTK inhibitor therapy in modulating clonal dynamics warrants consideration for completeness, the evidence does not support a causal relationship between zanubrutinib and AML in this case.

3.1. The Evidence for Clonal Evolution

The molecular evidence in this case provides compelling support for clonal evolution as the primary mechanism. Critically, the MCL and AML harbored molecularly distinct *TP53* abnormalities: the original MCL demonstrated a 17p deletion affecting the *TP53* locus, while the AML clone carries a *TP53* p.Tyr220Cys point mutation, a well-characterized pathogenic hotspot in the DNA-binding domain. These represent independent genetic events, definitively proving that the AML did not arise from direct transformation of the MCL clone. Furthermore, the original MCL clone was effectively eradicated, as evidenced by the disappearance of MCL-associated mutations (*ID3* and *BIRC3* decreasing from VAFs of 43% and 42% at diagnosis to undetectable levels at AML diagnosis) (Figure 1), resolution of the 17p deletion, absence of B-cell clonality, and complete metabolic response on PET-CT. However, both *TP53* abnormalities likely arose from a common *TP53*-altered ancestral hematopoietic progenitor, with the persistent MRD positivity during MCL remission representing this pre-leukemic clone rather than residual lymphoma.

The pre-leukemic clone most likely predated zanubrutinib therapy. Clonal hematopoiesis mutations such as *DNMT3A* and *TET2* are strongly age-associated, accumulating over years to decades in hematopoietic stem cells, and are present in over 10% of individuals older than 65 years. In our patient, who was in her late 80s, the probability of pre-existing clonal hematopoiesis was inherently high. Although myeloid-associated genes were negative at the 1% VAF detection threshold at MCL diagnosis, the pre-leukemic clone was likely present at sub-threshold levels and became detectable only after the MCL clone was eliminated, unmasking the myeloid clone in the peripheral blood compartment.

The seminal work by Wiegand et al. provides a mechanistic framework for understanding these observations and fundamentally challenges traditional models of lymphoma pathogenesis [4]. In their comprehensive analysis of B-cell non-Hodgkin lymphoma patients, they demonstrated that hematopoietic stem cells harbor premalignant clonal hematopoiesis mutations that can serve as the cellular origin of lymphoma. Critically, identical clonal hematopoiesis clones were detected in 41% of corresponding lymphomas, but these clones displayed distinct clonal dynamics depending on the mutated gene. *TP53*-mutant clones exhibited "tumor-promoting" behavior, showing expansion within the B-NHL compartment (10 of 16 clones), whereas *DNMT3A*-mutant clones demonstrated "tumor-infiltrating" behavior without expansion. This distinction is particularly relevant to our case, where the *TP53*-mutated clone expanded during MCL remission—consistent with the tumor-promoting phenotype described by Wiegand et al. Perhaps most strikingly, they identified lymphoma-associated mutations in flow-sorted hematopoietic progenitors from patients with indolent B-NHL (including follicular, mantle cell, and marginal zone lymphoma) but not in patients with aggressive B-NHL. Single-cell genotyping confirmed the presence of mutated progenitors in 2 mantle cell lymphoma patients specifically, providing direct evidence of a pre-neoplastic state in MCL pathogenesis [4]. Their observation of a stepwise accumulation of mutations along the lymphoid differentiation path suggests that MCL may originate from early hematopoietic progenitors rather than mature B cells, with additional mutations acquired during lymphoid differentiation.

TP53 mutations are present in only 5–10% of de novo AML cases, making the probability of two independent *TP53*-mutated malignancies arising by chance exceedingly low.

Landmark studies have fundamentally changed our understanding of therapy-related myeloid neoplasms by demonstrating that *TP53* mutations in t-AML are not induced by chemotherapy but rather represent pre-existing rare hematopoietic stem/progenitor cells that are resistant to therapy and expand preferentially after treatment [5,6]. In one pivotal study, the exact *TP53* mutation found at t-AML diagnosis was detected at low frequencies of 0.003–0.7% in samples obtained 3–6 years before t-AML development, including cases where *TP53* mutations were present before any chemotherapy was administered [5]. This patient's MCL harbored 17p deletion at diagnosis, and the emergence of a distinct *TP53* point mutation during remission indicates that a separate *TP53*-mutated clone was expanding—one that was never part of the lymphoma but shared a common ancestral origin.

The AML's mutational signature is highly consistent with transformation from *TP53*-mutated clonal hematopoiesis. *DNMT3A* mutations are the most common mutations in clonal hematopoiesis and frequently co-occur with *TP53* in therapy-related AML. In patients with clonal hematopoiesis who transformed to AML, four of six had *TP53* mutations at CH with expansion of the clone at transformation, and two acquired *NPM1* mutations while one acquired *FLT3* mutations—a pattern strikingly similar to our patient [6]. Recent mechanistic studies have confirmed that while monoallelic *TP53* mutations confer clonal fitness and therapy resistance, biallelic *TP53* mutations are essential for leukemic transformation, causing the genomic instability that permits acquisition of additional driver mutations [7].

The persistence of *KMT2C* p.Val961Ile at similar VAF (~8%) across all three timepoints—MCL diagnosis, remission, and AML—raises the intriguing possibility of a common hematopoietic ancestor and extends the findings of Wiegand et al. in a novel direction. Our case demonstrates that pre-existing clones can evolve independently toward myeloid malignancy while the lymphoid malignancy is successfully treated. The detection of a molecularly distinct *TP53* point mutation (p.Tyr220Cys) during MCL remission—separate from the original 17p deletion in the MCL—suggests that multiple *TP53*-altered clones may coexist within the hematopoietic compartment, each capable of independent evolution. The stable *KMT2C* VAF across MCL diagnosis, remission, and AML transformation suggests this mutation was present in a common ancestral hematopoietic clone that gave rise to both the lymphoid (MCL) and myeloid (AML) lineages, consistent with the shared clonal origins model proposed by Wiegand et al. [4]. *KMT2C* mutations are among the most frequent genetic lesions in AML with chromosome 7 abnormalities, affecting 16% of cases. Halik et al. demonstrated that *KMT2C* mutations frequently co-occur with *TP53* mutations in this AML subgroup, and cancer cell fraction analysis identified *TP53* mutations as mainly disease-initiating events [8]. Chen et al. showed that *KMT2C* deletions convey a selective advantage to hematopoietic stem cells after chemotherapy, enhancing self-renewal capacity without altering proliferation rates [9].

3.2. Contextualizing BTK Inhibitor Therapy and Second Malignancies

While the molecular evidence strongly favors clonal evolution, a complete discussion requires contextualizing the role of BTK inhibitor therapy. All BTK inhibitors carry warnings for second primary malignancies. All BTK inhibitors carry warnings for second primary malignancies, and the class effect cannot be dismissed. In pooled analyses of CLL patients receiving BTK inhibitors, second cancers occur in 9–22% of patients, with an observed-to-expected ratio of 2.2, indicating significantly elevated risk compared to the general population [1]. Acalabrutinib demonstrates a similar pattern, with second primary malignancies occurring in 12–18% of patients across pooled analyses of over 1000 patients [10]. In the ELEVATE-TN trial, second primary malignancies occurred in 11% of patients receiving acalabrutinib–obinutuzumab and 9% with acalabrutinib monotherapy, compared to 8%

with chlorambucil–obinutuzumab [11]. More recently, the AMPLIFY trial demonstrated that second primary cancers were more common with acalabrutinib–venetoclax combinations (4.2–5.2%) than with chemoimmunotherapy (0.8%), suggesting that prolonged BTK inhibitor exposure may contribute to malignancy risk through mechanisms distinct from chemotherapy-induced DNA damage [12]. However, the data from acalabrutinib studies reveal that only 1% of these second malignancies are hematologic, with the vast majority (55% in ELEVATE-TN) being non-melanoma skin cancers [11]. This pattern suggests that BTK inhibitors predominantly increase the risk of solid tumors and skin cancers rather than myeloid neoplasms. Perhaps most compellingly, the TRIANGLE trial demonstrated that secondary hematological malignancies (1 AML, 1 MDS) occurred only in the chemotherapy-alone arm, not in the ibrutinib-containing arms [3]. Similarly, the GAIA/CLL13 trial showed that second primary cancers occurred in 29% of patients receiving chemoimmunotherapy versus only 15–17% in venetoclax-based arms [12]. This strongly suggests that BTK inhibitors and targeted therapies are less leukemogenic than conventional chemotherapy, not more.

Several biological mechanisms could plausibly link BTK inhibitor therapy to myeloid leukemogenesis. BTK is expressed not only in B cells but also in myeloid cells, where it regulates innate immune responses, inflammasome activation, and phagocytosis. Chronic BTK inhibition causes profound immunomodulation, including reduced NK cell cytotoxicity, impaired T-cell function, and decreased immunoglobulin levels. This immunosuppressed state may permit expansion of pre-leukemic clones that would otherwise be eliminated by immune surveillance. In our patient, peripheral blood flow cytometry performed 3 months after zanubrutinib–rituximab initiation provided direct evidence of treatment-induced immune perturbation. Complete B-cell aplasia was observed (CD19 0%, CD20 0%, CD22 0%), consistent with the combined effect of rituximab-mediated B-cell depletion and BTK inhibition. Notably, the T-cell compartment demonstrated an inverted CD4:CD8 ratio (25% vs. 62%), suggesting CD4+ T-helper cell depletion. NK cells were present at low-normal levels (CD56 10%). This immunophenotypic profile—characterized by absent humoral immunity and impaired T-helper function—is consistent with a state of diminished immune surveillance that may have facilitated the permissive expansion of the pre-leukemic *TP53*-mutated clone.

The differential kinase selectivity of BTK inhibitors is relevant to understanding their potential impact on clonal dynamics. Ibrutinib, a first-generation BTK inhibitor, inhibits multiple off-target kinases including ITK, EGFR, TEC, BMX, and SRC family kinases, whereas zanubrutinib was specifically designed to minimize off-target inhibition of TEC- and EGFR family kinases and demonstrates significantly greater selectivity for BTK versus EGFR, FGR, FRK, HER2, HER4, ITK, JAK3, LCK, BLK, and TEC [13,14]. This differential selectivity has important immunological consequences. Ibrutinib's potent irreversible inhibition of ITK—a critical kinase in T-cell receptor signaling—drives a Th1-selective pressure in T lymphocytes and significantly alters T-cell homeostasis, including increased T-cell numbers, reduced activation-induced cell death, and decreased Treg/CD4+ ratios [15,16]. In contrast, zanubrutinib's minimal ITK inhibition is expected to preserve T-cell function more effectively, and clinical data suggest improved T-cell responses and vaccination efficacy with zanubrutinib compared to ibrutinib [17]. Paradoxically, ibrutinib's broader kinase inhibition profile may exert more widespread selective pressure on the hematopoietic compartment, potentially affecting myeloid progenitors more directly given that BTK is constitutively phosphorylated in AML blasts and ibrutinib inhibits AML blast proliferation at clinically relevant concentrations [18]. Zanubrutinib's greater selectivity may spare myeloid progenitors from direct kinase-mediated effects while still altering the competitive clonal landscape through B-cell depletion and immune remodeling. Whether

this differential selectivity translates into different risks of myeloid transformation remains unknown, but the absence of reported myeloid neoplasms with zanubrutinib—in contrast to the documented cases with ibrutinib—could reflect either this differential selectivity, shorter follow-up, or smaller patient populations.

BTK inhibitor therapy also exerts strong selective pressure on the hematopoietic compartment. Studies using droplet-microfluidic technology have demonstrated that ibrutinib-resistant subclones are detectable at low levels in pretreatment samples, indicating that therapy causes clonal selection and expansion rather than de novo mutagenesis. While this has been studied primarily for BTK-mutant CLL clones, the same principle could apply to *TP53*-mutated hematopoietic stem cells. In our patient, peripheral blood flow cytometry performed 3 months after zanubrutinib–rituximab initiation demonstrated complete B-cell aplasia (CD19 0%, CD20 0%, CD22 0%) and an inverted CD4:CD8 ratio (25% vs. 62%), consistent with diminished immune surveillance. By eliminating the MCL clone and altering the competitive hematopoietic landscape, zanubrutinib–rituximab may have unmasked a pre-existing myeloid clone that was previously below the detection threshold. However, this represents a permissive rather than causative role, and the myeloid clone may well have expanded to clinical significance regardless of the therapeutic agent used.

3.3. Integrating the Evidence: A Unified Model

The molecular evidence overwhelmingly favors clonal evolution from a pre-existing *TP53*-mutated hematopoietic progenitor as the primary mechanism. The pre-leukemic clone most likely predated zanubrutinib therapy, given that clonal hematopoiesis mutations such as *DNMT3A* and *TET2* are age-related and typically accumulate over years to decades, and the patient's advanced age placed her at inherently high baseline risk for clonal hematopoiesis. The longitudinal molecular data suggest a model wherein a *KMT2C*-mutant ancestral hematopoietic clone gave rise to two distinct lineages (Figure 2): one acquiring *IGH::CCND1*, *ID3*, *BIRC3*, and 17p deletion to become MCL, and another acquiring *DNMT3A*, *TET2*, *TP53* p.Tyr220Cys, and eventually *FLT3-TKD* to become AML. The distinct *TP53* abnormalities in MCL (17p deletion) versus AML (p.Tyr220Cys point mutation), the mutational profile consistent with CH-derived AML, and the dramatically lower t-MN rates with BTK inhibitors compared to chemotherapy all point toward disease biology rather than drug toxicity. The successful treatment of MCL with zanubrutinib–rituximab eliminated the lymphoid clone—as evidenced by the dramatic reduction in *ID3* and *BIRC3* VAFs from 42.62% and 42.09% to 0.09% and 0.08%, respectively—while the myeloid clone, potentially present at low levels before treatment, expanded to clinical significance.

However, zanubrutinib therapy may have contributed indirectly by creating selective pressure favoring expansion of the *TP53*-mutated clone, inducing immunosuppression that impaired surveillance against the emerging leukemic cells, and eliminating competing clones to provide a fitness advantage to the *TP53*-mutated progenitor. Whether zanubrutinib actively promoted this expansion or whether the myeloid clone would have emerged regardless of treatment cannot be definitively determined. The detection of the pre-leukemic clone during remission—before prolonged zanubrutinib exposure—suggests the clone was expanding independently of therapy. However, the possibility that BTK inhibition altered the competitive dynamics between clones cannot be excluded. In this model, zanubrutinib did not cause the AML through direct mutagenesis but may have facilitated its emergence by altering the competitive landscape of hematopoiesis.

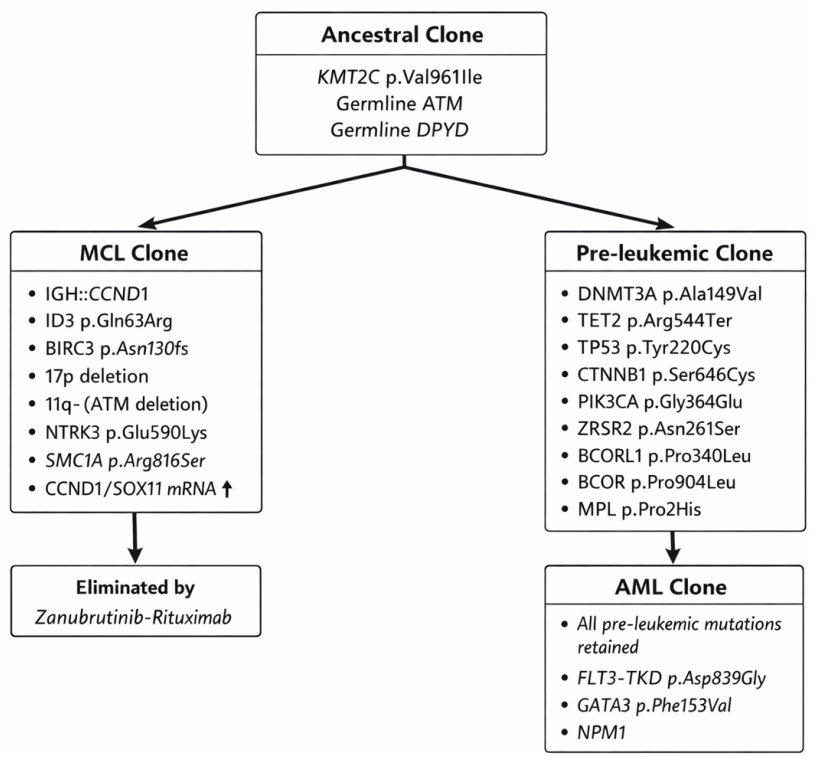


Figure 2. Proposed model of clonal evolution demonstrating divergent hematologic malignancies arising from a common ancestral clone.

The presumed germline *ATM* mutation (p.Lys468GlufsTer18) in our patient may have contributed to genomic instability facilitating both MCL development and subsequent myeloid transformation. *ATM* is a master regulator of the DNA damage response, and germline *ATM* mutations are associated with increased cancer susceptibility. The presence of somatic second-hit *ATM* inactivation (11q deletion) in the tumor tissue, combined with germline heterozygosity, created biallelic *ATM* loss within the MCL clone—a recognized high-risk feature that may have predisposed to the development of multiple malignancies.

4. Methodological Considerations

MRD monitoring in this case was performed using serial peripheral blood NGS rather than conventional MCL-specific assays such as ClonoSeq. While tracking *TP53* mutation VAF is not standard practice for MCL MRD monitoring, the comprehensive NGS approach fortuitously captured the emerging myeloid clone. This case illustrates the potential value of broad molecular surveillance in high-risk lymphoma patients, as it may identify pre-leukemic clones before clinical transformation.

Several additional methodological limitations should be acknowledged. First, the germline nature of the *ATM* p.Lys468GlufsTer18 mutation was inferred from its consistently high VAF across independent samples (77% in tissue, 69% in peripheral blood) but was not confirmed by testing of non-hematopoietic tissue (e.g., buccal swab or skin fibroblasts). While the high VAF and concordance across sample types strongly suggest germline origin, formal confirmation would require paired germline testing. Second, several variants reported in this case—including *ID3* (VAF 0.09%), *BIRC3* (VAF 0.08%), *BCORL1* (VAF 0.92% and 0.78%), *BCOR* (VAF 0.75% and 0.9%), *MPL* (VAF 0.66% and 0.26%), *FLT3-TKD* (VAF 0.87%), and *TET2* at AML diagnosis (VAF 0.03%)—fall below the assay's validated 1% VAF sensitivity threshold. These sub-threshold variants are flagged in Table 1 and should be interpreted with caution, as they may be subject to sequencing noise. However, the biological plausibility of these findings—particularly the concordance of sub-threshold

MCL markers (*ID3*, *BIRC3*) with clinical remission and the emergence of *FLT3-TKD* as a known AML driver—supports their clinical relevance. Third, the absence of a baseline (pre-treatment) peripheral blood NGS specifically interrogating myeloid genes at high sensitivity limits the ability to definitively determine whether the CH clone was present before zanubrutinib exposure. While myeloid-associated genes were included in the diagnostic panel and were negative at the 1% threshold, the pre-leukemic clone may have been present at sub-threshold levels. Finally, quantitative immunoglobulin levels were not monitored during treatment, which would have provided additional characterization of the degree of humoral immunosuppression.

5. Conclusions

This case demonstrates the power of longitudinal molecular tracking in distinguishing clonal evolution from therapy-related malignancy. The identification of molecularly distinct *TP53* abnormalities in MCL (17p deletion) versus AML (p.Tyr220Cys point mutation), combined with real-time capture of a pre-leukemic clone during remission, provides compelling evidence that AML arose from evolution of a pre-existing hematopoietic clone rather than direct MCL transformation or therapy-related mutagenesis. The shared *KMT2C* mutation across all disease states raises the possibility of a common ancestral clone, consistent with recent evidence from Wiegand et al. demonstrating shared clonal origins in B-cell lymphomas [4]. Importantly, this case should not be interpreted as establishing a causal association between zanubrutinib and secondary AML; rather, it underscores the need for molecular vigilance in patients with high-risk genomic features—particularly germline *ATM* mutations and *TP53* aberrations—regardless of therapeutic agent. To our knowledge, this is the first report providing serial NGS documentation of real-time pre-leukemic myeloid clone emergence during BTK inhibitor therapy for MCL, highlighting the value of broad-panel molecular surveillance over conventional imaging-based response assessment in high-risk hematologic malignancies. As BTK inhibitors become standard therapy for MCL, serial NGS monitoring may identify patients at risk for myeloid transformation, potentially enabling early intervention strategies.

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