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Clonal origin tracing and integrated biomarker analysis in a *JAK2V617F*-positive essential thrombocythemia patient progressing to T-cell acute lymphoblastic leukemia

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Running heads: Clonal Evolution in ET to T-ALL Transformation

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Contributions

YZ and GZ designed the research, interpreted the data, and wrote and revised the manuscript. YW and DT conducted and coordinated the research, analyzed the data and wrote the manuscript. ZC and LD analyzed the data and commented on the manuscript. All authors had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis and approved the final version to be published.

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Disclosures

No potential conflict of interest was reported by the authors.

Main text

Transformation of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) into acute leukemia is a well-known but devastating complication that dramatically worsens prognosis.[1] The majority of such transformations result in acute myeloid leukemia (AML); secondary lymphoid transformation, particularly to T-cell acute lymphoblastic leukemia (T-ALL), remains exceptionally rare.[2] Only a few dozen such cases have been reported,[3] and the molecular mechanisms underpinning lineage divergence remain poorly understood. Here, we describe a patient with JAK2V617F-positive essential thrombocythemia (ET) who developed T-ALL within two years. Through integrative genomic, transcriptomic, and proteomic analyses, we identified independent myeloid and lymphoid clones arising from a shared clonal hematopoiesis background. This case highlights the biological complexity of MPN evolution and underscores the clinical importance of early genomic surveillance. The study was approved by the Institutional Ethics Committee of the Second Xiangya Hospital, Central South University (Approval No. LYEC2024-0082).

A 54-year-old man with a two-year history of *JAK2V617F*-positive essential thrombocythemia (ET) developed lymphadenopathy and systemic symptoms suggestive of malignant transformation. His ET had initially been characterized by increased megakaryocytes with atypical morphology and an elevated platelet count ranging from 732×10□/L to 1351×10□/L. Treatment with intermittent α-interferon and aspirin maintained stable counts until two years after diagnosis, when the patient presented with oropharyngeal discomfort, cervical lymphadenopathy, and night sweats. Lymph node biopsy revealed a T-lymphoblastic lymphoma phenotype positive for CD3, CD5, and CD10, with a Ki-67 index of 50%.

On admission, PET-CT revealed multiple hypermetabolic cervical and supraclavicular lymph nodes (SUVmax 26.6). Bone marrow aspirate demonstrated hypercellularity with 62% lymphoblasts, confirming progression to T-cell acute lymphoblastic leukemia (T-ALL). Flow cytometry identified a dominant population (70.6%) of immature T-lymphoblasts expressing CD5, CD7, cCD3, CD34, TdT, and

CD38, consistent with T-ALL, while surface CD3, CD4, CD8, and MPO were negative. The *JAK2V617F* mutant: wild-type ratio was elevated at 366%. The patient's karyotype was normal 46, XY, and BCR-ABL1 transcripts were absent.

The patient was treated with hyper-CVAD chemotherapy combined with Ruxolitinib (10 mg twice daily). After one cycle, marrow blasts decreased from 62% to 20%. Whole-exome sequencing (WES) result showed the presence of DNMT3A mutations, suggesting underlying epigenetic dysregulation and potential sensitivity to hypomethylating agents. Therefore, we added azacitidine (100 mg/day, days 1-7) during the second cycle, which further reduced blasts to 17%. Following a third cycle incorporating high-dose methotrexate and cytarabine plus azacitidine, complete remission was achieved (4% blasts). Consolidation with VIDCP was followed by haploidentical allogeneic hematopoietic stem cell transplantation (HSCT), resulting in durable remission.

Comprehensive molecular profiling was undertaken to delineate the clonal origin of the neoplasm. WES identified pathogenic variants in *DNMT3A*, *IKZF1*, *NOTCH1*, and *CSF3R*, alongside *JAK2V617F*. Specifically, *IKZF1* p.Arg83*, *DNMT3A* p.Gly869Ser and splice-site mutation (c.855+2T>C), and *CSF3R* p.Thr618Ile and p.Lys785Ser*26 were detected. The variant allele frequencies (VAFs) for *DNMT3A* and *IKZF1* were approximately 25%, while *JAK2V617F* showed a VAF of 13%. These results suggested coexistence of distinct myeloid and lymphoid mutational signatures.

To investigate lineage-specific origins, bone marrow mononuclear cells were fractionated by immunomagnetic sorting into CD7□ lymphoid, CD13□ myeloid, and CD7□CD13□ compartments. Sanger sequencing revealed that CD13□ myeloid cells harbored only *JAK2V617F*, whereas CD7□ lymphoid cells carried *DNMT3A*, *IKZF1*, and *CSF3R* mutations but lacked *JAK2V617F* (Figure 1.A-F). Combining with WES, immunomagnetic sorting and Longitudinal RNA sequencing, the findings demonstrated that the ET and T-ALL clones arose from independent progenitors, establishing clonal divergence rather than direct transformation.

Longitudinal RNA sequencing provided dynamic insight into clonal behavior. At diagnosis, transcripts associated with the T-ALL clone (*IKZF1*, *DNMT3A*, *NOTCH1*, and *CSF3R*) were abundant but declined markedly after chemotherapy, consistent with suppression of the lymphoid clone (Table 1). In contrast, *JAK2V617F* transcript levels remained stable throughout treatment (23.85% at diagnosis; 16.36% after two cycles; 27.25% post-remission) (Figure 1.G), confirming persistence of the original myeloproliferative clone despite hematologic remission. This pattern suggests a competitive "seesaw" model between coexisting myeloid and lymphoid clones, wherein therapeutic suppression of one population allows expansion of the other.

Western blot analysis elucidated the functional effects of therapy on signaling pathways. At baseline, JAK2, STAT3, and STAT5 were strongly phosphorylated, indicating constitutive JAK/STAT activation (Figure 1.H). After two cycles of hyper-CVAD plus Ruxolitinib, phosphorylated JAK2, STAT3, and STAT5 became undetectable, with further reductions in total protein levels following additional therapy. These findings confirmed effective biochemical suppression of the JAK/STAT pathway, although incomplete remission after early cycles suggested that pathway inhibition alone was insufficient to eradicate the dominant T-ALL clone.

The coexistence of *JAK2V617F*-positive ET and subsequent T-ALL raises important questions about hematopoietic clonal evolution. Transformation of Philadelphia chromosome-negative myeloproliferative neoplasms typically results in acute myeloid leukemia,[1, 4-7] while lymphoid transformation remains exceedingly rare.[2] A recent review identified only 31 such cases, with just five involving T-lineage leukemia [3]. The rapid onset of T-ALL within two years in this patient contrasts with the median latency of nearly a decade reported in most series,[3, 8] implying either pre-existing clonal hematopoiesis (CH) or selective pressure favoring lymphoid expansion.

Recent studies have increasingly implicated CH as a contributor to the development of secondary hematologic malignancies, including ALL. CH, defined by the expansion of hematopoietic stem/progenitor cells (HSPCs) harboring somatic driver

mutations, is a well-established precursor state for myeloid neoplasms but is now also being recognized in the context of lymphoid transformation.[9] CH-associated mutations in genes such as *DNMT3A*, *TP53*, and other epigenetic regulators have been detected in individuals long before the onset of overt ALL, and these mutations can persist through therapy and relapse, suggesting a multipotent founder clone with lymphoid and myeloid potential.[10, 11] In large adult ALL cohorts, CH-associated myeloid mutations were shown to predate ALL diagnosis by several years and were associated with persistent clonal signatures through remission and relapse, indicating that CH may provide a genetic substrate for leukemogenesis in the lymphoid lineage.[10] Moreover, studies of patients with antecedent myeloid neoplasms have reported molecular evidence of CH clones contributing to divergent clonal evolution, whereby a shared preleukemic clone gives rise to distinct myeloid and lymphoid malignancies, including rare cases of secondary ALL after myeloproliferative neoplasms.[3] These observations underscore the role of CH not only as a risk factor for hematologic malignancy broadly but also as a mechanistic link in the pathogenesis of secondary ALL arising from a preexisting clonal hematopoietic architecture.

The mutational architecture in this case supports a model of parallel evolution from a shared hematopoietic progenitor. *DNMT3A* mutations - frequent in CH and T-ALL[12, 13] - likely arose early, establishing a preleukemic lymphoid-prone clone. The subsequent acquisition of *IKZF1, NOTCH1* and *CSF3R* mutations may have driven full leukemic transformation, independent of the *JAK2V617F* clone responsible for ET. This model aligns with recent reports showing that *DNMT3A* mutations often predate *JAK2V617F* in myeloproliferative neoplasms and persist after secondary leukemia,[14] implicating them as founder lesions within a shared progenitor compartment. The persistence of *JAK2V617F* after T-ALL remission further supports its clonal independence.

The therapeutic implications are significant. Ruxolitinib effectively inhibited JAK/STAT phosphorylation but failed to eliminate the lymphoid clone, while the addition of azacitidine enhanced chemosensitivity, enabling remission. This suggests

that epigenetic dysregulation via *DNMT3A* mutation may contribute to chemoresistance and that hypomethylating therapy can re-sensitize refractory lymphoid blasts. Ultimately, allogeneic HSCT provided curative potential by eradicating both malignant clones. Given the poor prognosis of MPN-to-ALL transformation, early transplantation remains the most rational strategy for achieving durable remission.

This case highlights the necessity of integrative molecular monitoring in MPN patients who develop atypical lymphoid features. Multilineage sequencing and transcriptomic tracking revealed two coexisting malignant populations with distinct origins (Figure 2). The dynamic interplay between these clones underscores the plasticity of CH and the importance of early genomic surveillance in identifying emergent leukemic evolution.

In conclusion, T-ALL developing in a *JAK2V617F*-positive ET patient arose from an independent lymphoid clone rather than direct transformation of the myeloproliferative clone. The coexistence of *DNMT3A*, *IKZF1*, and *CSF3R* mutations within the T-ALL population and persistence of *JAK2V617F* expression in remission support a model of parallel clonal evolution from a shared preleukemic stem cell. Therapeutic suppression of JAK/STAT signaling with Ruxolitinib and epigenetic reprogramming with azacitidine, followed by HSCT, achieved long-term remission. This case provides mechanistic insight into lymphoid transformation within a background of clonal hematopoiesis and reinforces the clinical value of integrative genomic analysis in myeloproliferative neoplasms.

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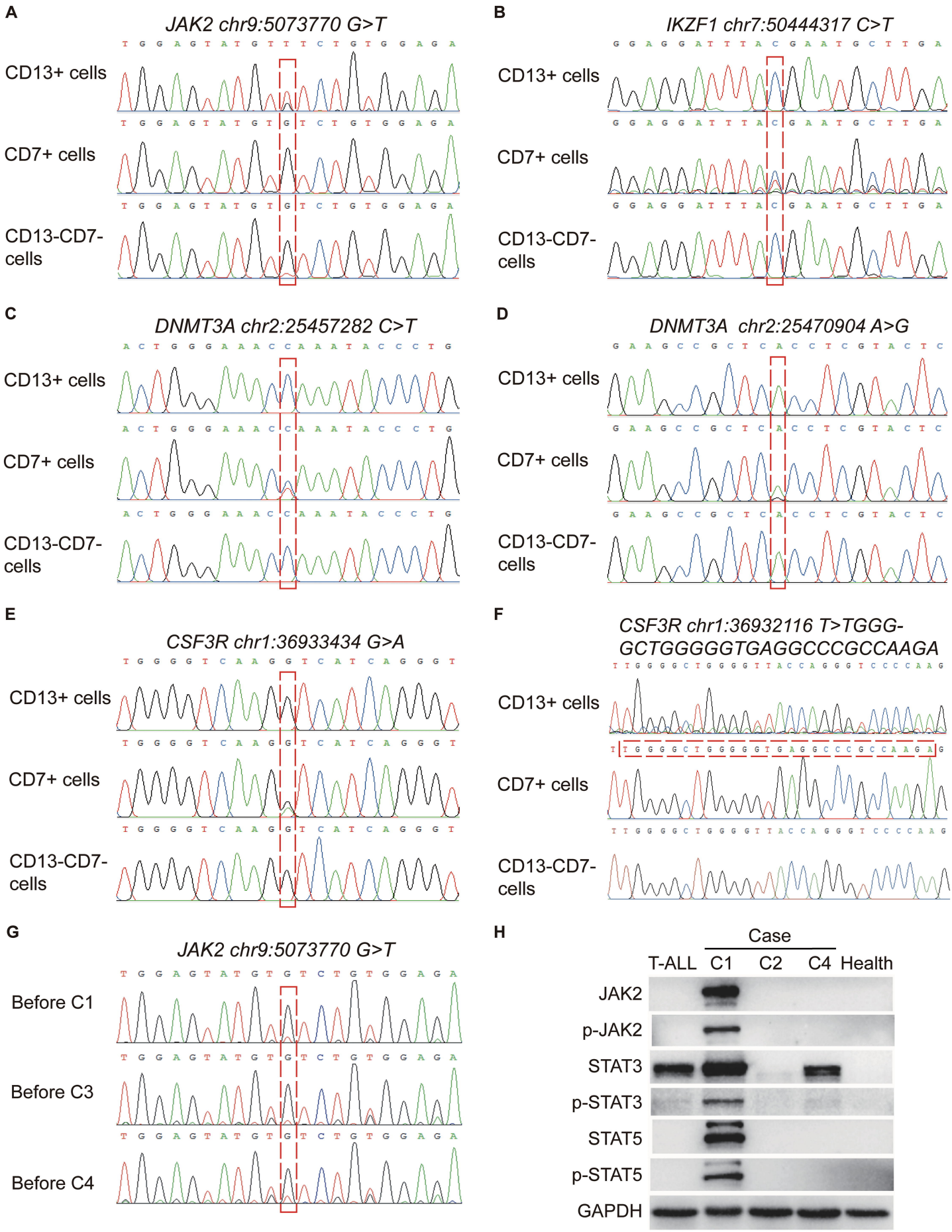
Table 1. RNA-seq analysis on the expression abundances of mutated genes before and after treatment in bone marrow.

Gene	Chr.	Location	Mutant nucleotide abundances (%)		
			Before C1	Before C3	Before C4
JAK2	chr9	5073770	23.85321101	16.36363636	27.25060827
IKZF1	chr7	50444317	19.65601966	3.921568627	0.547945205
DNMT3A	chr2	25457282	49.66666667	50	10.16949153
DMMT3A	chr2	25470904	40.6779661	26.66666667	20.83333333
NOTCH1	chr9	139399365	1.6	10	0
CSF3R	chr1	36933434	16.47940075	9.321058688	0.531208499

Figure legends

Figure 1. Lineage-specific distribution of *JAK2, IKZF1, DNMT3A,* and *CSF3R* mutations and dynamic JAK-STAT pathway activation during treatment. Sanger sequencing results on isolated $CD7^+$ or $CD13^+$ or $CD13^-$ $CD7^-$ cells for *JAK2*, *IKZF1*, *DNMT3A* and *CSF3R* genes mutation (A-F). (A) *JAK2V617F* mutation in $CD13^+$ cells are positive, but could not be found in CD7□ cells. (B) *IKZF1* mutation (chr7:50444317 C>T) (hg19) was only detected in CD7□ cells. (C-D) *DNMT3A* mutations (chr2:25457282 C>T and chr2:25470904 A>G) were only found in CD7□ cells. (E-F) *CSF3R* mutations (chr1:36933434 G>A and chr1:36932116T>TGGGGCTGGGGGTGAGGCCCGCCAAGA) were only detected in CD7□ cells. (G) *JAK2V617F* mutation detection in bone marrow before and after chemotherapy. Before C1: before first cycle chemotherapy (pre-treatment); Before C3: after two cycle chemotherapy and before third cycle chemotherapy; Before C4: before fourth cycle chemotherapy. (H) JAK-STAT signaling pathway activation in serial bone marrow samples. C1: diagnostic sample (pre-treatment); C2: Pre-cycle 2 (post-hyper-CVAD + Ruxolitinib); C4: Pre-cycle 4 chemotherapy; T-ALL: Control sample from a *JAK2 V617F*-negative T-cell acute lymphoblastic leukemia patient. Health: Healthy donor marrow. GAPDH: Loading control.

Figure 2. Schematic diagram of ET and sT-ALL clone formation and independent evolution based on clonal hematopoiesis-variation trajectory of molecular markers before and after chemotherapy. 1. Clonal Hematopoiesis originating from a *DNMT3A* mutation-driven cell population. 2. Following the first hit to these hematopoietic cells, a *JAK2V617F* mutant subclone emerges (*JAK2* mut (+) cells/*DNMT3A* mut (+) cells). 3. Upon a second hit to the *DNMT3A* mut (+) background cells, a dominant T-ALL clone develops, characterized by molecular markers *NOTCH1/IKZF1/CSF3R* mutations. 4. After effective therapy, the dominant T-ALL clone regresses (evidenced by significant downregulation of *NOTCH1/IKZF1/CSF3R* expression), while the *JAK2V617F/DNMT3A* mut (+) clone rebounds (showing upregulated expression).


A
JAK2 chr9:5073770 G>T
CD13+ cells
CD7+ cells
CD13-CD7- cells
B
IKZF1 chr7:50444317 C>T
CD13+ cells
CD7+ cells
CD13-CD7- cells
C
DNMT3A chr2:25457282 C>T
CD13+ cells
CD7+ cells
CD13-CD7- cells
D
DNMT3A chr2:25470904 A>G
CD13+ cells
CD7+ cells
CD13-CD7- cells
E
CSF3R chr1:36933434 G>A
CD13+ cells
CD7+ cells
CD13-CD7- cells
F
CSF3R chr1:36932116 T>TGGG-GCTGGGGGTGAGGCCCGCCAAGA
CD13+ cells
CD7+ cells
CD13-CD7- cells
G
JAK2 chr9:5073770 G>T
Before C1
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Case
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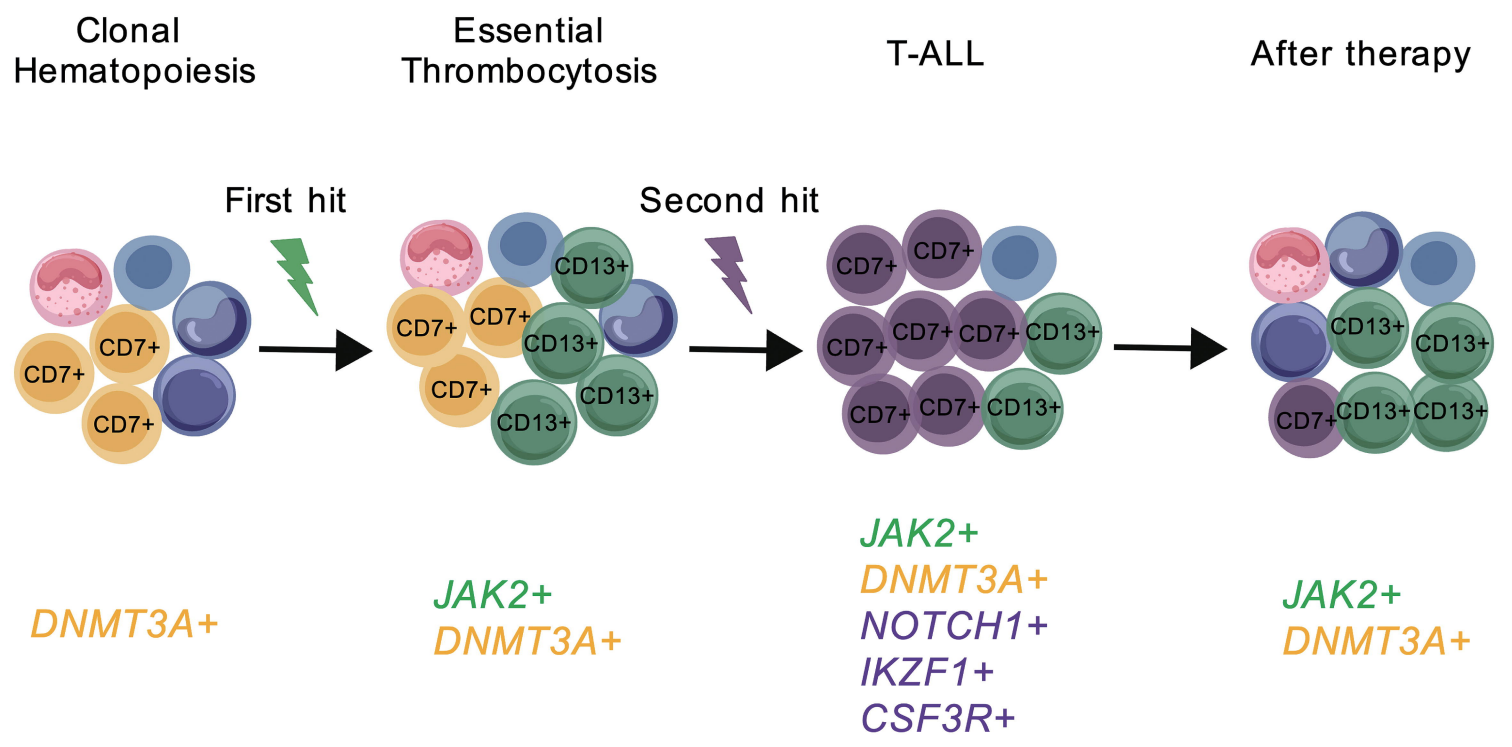
C4
Health
JAK2
p-JAK2
STAT3
p-STAT3
STAT5
p-STAT5
GAPDH


Clonal Hematopoiesis
Essential Thrombocytosis
T-ALL
After therapy
First hit
Second hit
CD7+
CD13+
DNMT3A+
JAK2+
DNMT3A+
JAK2+
DNMT3A+
NOTCH1+
IKZF1+
CSF3R+
JAK2+
DNMT3A+