Unveiling Myeloid Transformation: T-LGLL with Eosinophilia Masking Myeloid-Associated *STAT5B* Mutation Culminating in AML

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TO THE EDITOR:

The transcription factor signal transducer and activator of transcription 5B (STAT5B) plays a critical role as a downstream mediator of various growth factors and oncogenes[1,2].

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The *STAT5B* N642H mutation is recognized as a gain of function mutation[3-5] and is predominantly observed in lymphoid neoplasms initially, such as T cell large granular lymphocytic leukemia (**T-LGLL**) and T acute lymphoblastic leukemia[1,6-10]. Recent emerging evidence has indicated occurrences of *STAT5B* N642H in myeloid neoplasms, both with or without eosinophilia[6,11,12]. In lymphoid neoplasms, concomitant mutations are infrequent. In contrast, myeloid neoplasms with *STAT5B* mutations show more intricate genetic profiles, marked by a higher diversity and number of concurrent mutations[11,13]. However, discerning whether the *STAT5B* mutation originates in the lymphoid or myeloid lineage presents challenges in some cases. This report presents a distinctive scenario that began with an initial diagnosis of T-LGLL with eosinophilia, where the *STAT5B* mutated myeloid population remained obscured. Subsequently, disease progression led to the development of acute myeloid leukemia (**AML**) originating specifically from the *STAT5B* mutated myeloid cells.

A 67-year-old woman presented with anemia (hemoglobin 10.9g/dL) and thrombocytopenia (platelet 68 x10^9/L). Her white cell count was 6.5x10^9/L and showed neutropenia, eosinophilia, and lymphocytosis. The differential was: neutrophils 0.82 x10^9/L, 12.5%; lymphocytes 3.66 x10^9/L, 56.2%; monocyte 0.7 x10^9/L, 10.8%; eosinophils 1.25 x10^9/L, 19.2%; basophils 0.03 x10^9/L, 0.5%; immature granulocyte 0.05 x10^9/L, 0.7%). Peripheral blood smear analysis depicted increased eosinophils and small lymphocytes displaying irregular nuclear contours, condensed chromatin, and cytoplasmic granules (Figure 1A). Peripheral blood flow cytometry identified aberrant T cells (27% of total cells, CD3+, CD4−, CD8+, CD7dim/−, CD5dim/−) without Vbeta expression, consistent with T-LGLL (Figure 1B). T cell clonality was confirmed by gene rearrangement analysis. The in-house 30-gene lymphoid NGS analysis of peripheral blood detected a *STAT5B* p.N642H mutation with a variant allele frequency (**VAF**) of 26%. A bone marrow biopsy showed 80% cellularity, displaying increased eosinophils, mild megakaryocytic dysplasia (Figure 1C), and 10% abnormal T cells with similar immunophenotype as peripheral blood. Blasts were not increased by manual count or CD34 immunohistochemical staining (Figure 1D). Due to the hypercellularity and atypical megakaryocytes, a myeloid NGS (*STAT5B* gene was not covered) analysis was conducted on the bone marrow aspirate, revealing several somatic mutations: *PHF6* p. I314T (VAF 27%), *TET2* p.M1333Nfs*6 (VAF 27%), *TET2* p.E1413* (VAF 14%), and *TET2* splice site (VAF 30%). The karyotype was normal: 46, XX [20], and FISH for *PDGFRA*, *PDGRB*, *FGFR1*, *ETV6*, and *ABL1* rearrangements yielded negative results. The final diagnosis was T-LGLL with eosinophilia and clonal cytopenia of undetermined significance. The patient received methotrexate treatment.

Four years later, she presented with anemia, thrombocytopenia, and significantly increased eosinophilia (49%, absolute count 1960/μl), accompanied by occasional circulating myeloblasts (Figure 1E). A bone marrow biopsy revealed a hypercellular marrow (Figure 1F) with 15% myeloblasts on a suboptimal aspirate (Figure 1G), confirmed by CD34 immunohistochemical staining (Figure 1H). By flow cytometry, myeloblasts accounted for 20-25% of total cells and were CD34+, CD117+, CD38+, HLA−DR+. Additionally, a small aberrant B cell population (negative for CD5, CD10, and surface light chains, <1% of total cells), and a tiny clonal T cell population (positive for CD4 and CD8dim, <0.5% of

total cells) were detected. Molecular NGS studies revealed the previously detected *STAT5B* p.N642H (VAF 26%), *TET2* p.M1333Nfs*6 (VAF 41%), *PHF6* p.I314T (VAF 41%), and *TET2* splice site (VAF 42%) mutations, with several additional mutations, including *RUNX1* p.S388Ffs*167 (VAF 11%), *NRAS* p.A59delA (VAF 4%), and *U2AF1* p.34F (VAF 3%). *TET2* p.E1413*, which was detected in the previous bone marrow, was not identified. Cytogenetic analysis revealed an abnormal karyotype: 47, XX, +8 [3]/46, XX [17]. The patient was diagnosed with AML with eosinophilia and passed away shortly after the diagnosis.

To elucidate the clonal relationships and evolution among distinct cell populations, we performed comprehensive lymphoid and myeloid NGS analyses using genomic DNAs isolated from flow cytometry sort-purified bone marrow cells collected in 2022: CD34+ AML blasts, CD33+CD34− non-AML myeloid cells, CD3+ T cells, and CD19+ B cells (Figure 2A). The examination of shared and distinct somatic mutations among these populations (Figure 2B) along with newly detected trisomy 8 unveiled the clonal relationship between these populations (Figure 2C). Notably, mutations including *TET2* p.M1333Nfs*6, *PHF6* p.I314T, and *TET2* splice site were found shared among AML blasts, non-AML myeloid cells, and B cells, indicating these cells derived from a common precursor – likely a B-myeloid precursor. The presence of *STAT5B* mutation in both AML myeloblasts and non-AML myeloid cells suggests its emergence after myeloid commitment from B-myeloid precursors, further driven by newly acquired *RUNX1* mutation and trisomy 8, contributing to AML development. Conversely, the absence of *NRAS* and *U2AF1* mutations in any of these four populations retrospectively suggests their potential presence in the CD34−CD33− cells (purple fraction in Figure 2A), which unfortunately were not collected. Intriguingly, *STAT5B* mutation was not detected in the sorted T cells, which exclusively contained the *TET2* p.E1413* mutation, implying persistence of the original T cell clone. The absence of this mutation in unsorted cells is probably attributable to the low frequency of T cells in this marrow sample. The predominance of the T cell clone in the initial specimen likely masked the presence of the myeloid clone carrying the *STAT5B* mutation, thereby leading to the interpretation associating the *STAT5B* mutation with T cell-related eosinophilia in T-LGLL. Furthermore, the cell sorting uncovered *TNFAIP3* and *ZRSR* mutations solely in B cells suggesting a clone emerged post B cell commitment from the B-myeloid precursors.

Research has elucidated that *STAT5B* but not *STAT5A* can drive the self-renewal of hematopoietic and leukemia stem cells. This distinction clarifies why *STAT5B* is preferentially activated downstream of several oncogenes, leading to the frequent occurrence of gain of function mutations in STAT5B[2]. To explore whether a *STAT5B* mutation alone could transform hematopoietic stem and progenitor cells (**HSPCs**) into AML or necessitates cooperation with other mutations, such as *TET2* mutation, an animal experiment was conducted using control and *TET2* knockout (KO) mice, along with *STAT5B* retroviral transduction (Figure 2D). Remarkably, recipient mice injected with either wild type (WT) or N642H *STAT5B* transduced HSPCs developed AML within 3-7 weeks, irrespective of *TET2* deletion (Figure 2E). Notably, all recipient mice, whether transplanted with Control or TET2KO HSPCs, and expressing either WT or N642H STAT5B, develop myeloid leukemia with a consistent immunophenotype co-expressing myeloid markers Gr1 and CD11b.

However, the combined deletion of *TET2* and presence of *STAT5B* N642 demonstrated more rapid induction of AML (Figure 2F). These findings substantiate the principle that *STAT5B* alone can induce myeloid leukemia, and the simultaneous occurrence of somatic mutations enhances this process.

In this case, the pivotal question revolved around whether T-LGLL and the subsequent AML shared the *STAT5B* mutation and were clonally related. Typically, most diagnosticians may assume that the two diseases are related. However, our study, conducted through flow sort-purified cell populations, demonstrated the absence of a clonal relationship between the T and myeloid cells. These findings align with recent research indicating the rarity of the *STAT5B* mutation in CD8+ T-LGLLs compared to myeloid neoplasms[11,13] and CD4+ T-LGLL[9,14]. However, the absence of the *STAT5B* N642H mutation on sorted T-cells may be attributed to the lack of the prior T-LGL, and thus, the possibility of the STAT5B N642H mutation in T-LGL of the initial specimen cannot be completely ruled out. Nonetheless, transgenic mice expressing *STAT5B* N642H under the Vav1 promoter, expected to express *STAT5B* mutant in all hematopoietic cells, develop CD8+ T lymphocytic leukemia/lymphoma[5]. This, combined with instances where *STAT5B* mutation occurs in T, B, myeloid, and eosinophils[4], suggests that the same mutation in different cell lineage at distinct developmental stage with varying co-mutations may induce diverse types of neoplasms. It is important to note that the expression levels of *STAT5B* are also a key factor, as even the overexpression of WT *STAT5B* alone can induce myeloid leukemia. Further research endeavors are warranted to unravel the pathogenesis of *STAT5B*-mutated neoplasms and justify the development of specific *STAT5B* inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

FUNDING SOURCES

DATA AVAILABILITY STATEMENT

The data of this study are available on request from the corresponding author, C.Z.

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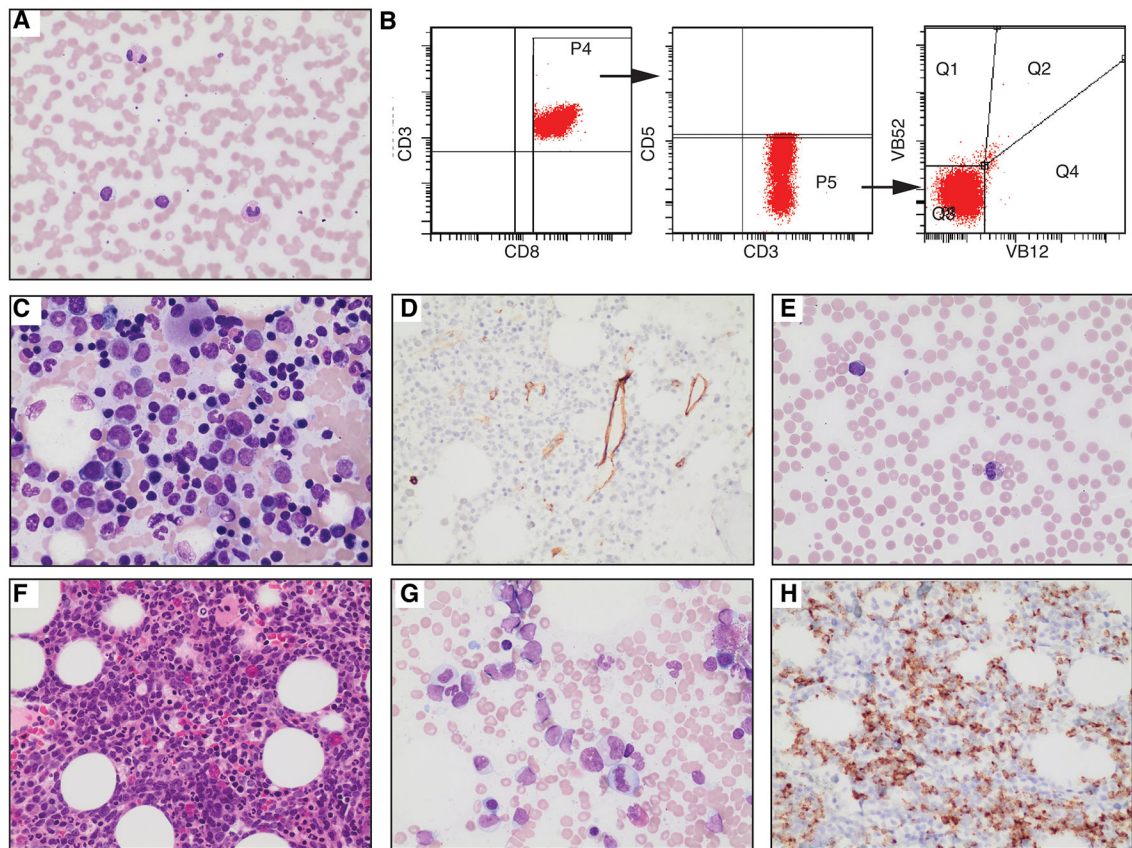
Figure 1. Morphological and immunophenotypic characterization of T-LGLL and Subsequent AML

A-D, samples from 2018 specimens. **E-H**, samples from 2022 specimens. (**A**) Peripheral blood smear of T-LGLL (Giemsa stain, 500X). Increased eosinophils and small, atypical lymphocytes with cytoplasmic granules are observed. (**B**) Flow cytometry of the patient's peripheral blood showed atypical T cells [CD3+, CD8+, CD5dim/−, CD7dim/− (not shown), and Vbeta-]. (**C**) Bone marrow aspirate (C: 500X) shows scattered small lymphocytes, increased eosinophils, and dysplastic megakaryocytes. (**D**) Immunohistochemical staining for CD34 (400X). (**E**) Peripheral blood smear (Giemsa stain, 500X) showing increased eosinophils and occasional circulating myeloblasts. (**F**) Bone marrow core biopsy (400X) exhibiting hypercellularity with a left shift. (**G**) Bone marrow aspirate (500X) indicating elevated myeloblasts. (**H**) CD34 immunostaining of the bone marrow core (400X).
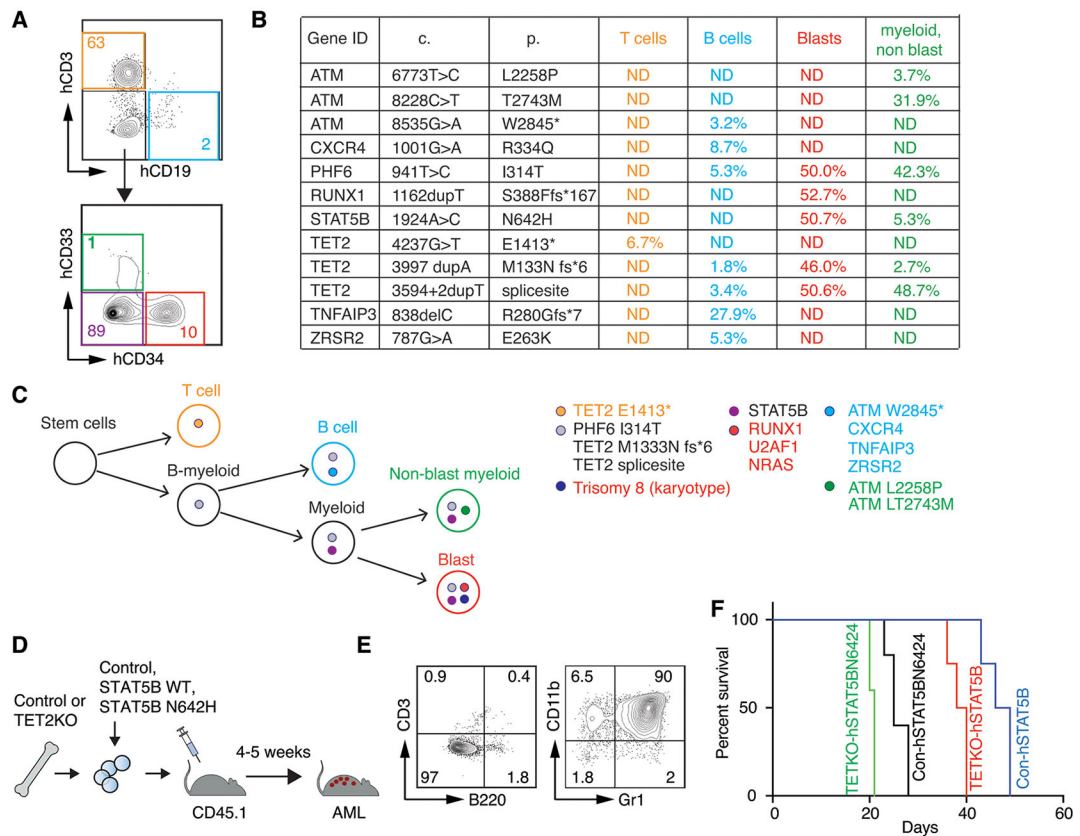
Figure 2. Clonal evolution of STAT5B mutated myeloid neoplasm and its role in leukemogenesis.
(**A**) Flow cytometry sorting strategy highlighting different cell populations labeled by various colors. (**B**) Summary of mutations detected in each cell population using an in-house myeloid and lymphoid NGS panel. (**C**) Hypothesized clonal evolution steps based on NGS mutational testing results from (**B**). (**D**) Schematic representation of the investigation into the role of STAT5B overexpression in leukemogenesis in a mouse model. Mouse hematopoietic stem/progenitor cells (HSPCs) were isolated from the bone marrows of control or TET2 knockout mice, then transduced with MSCV-IRES-GFP based retroviruses carrying an empty vector (Con), wild type (WT) or STAT5B N642H, and subsequently injected into lethally irradiated recipient mice. (**E**) Representative flow cytometry profile illustrating the development of myeloid leukemia induced by both WT and N642H STAT5B overexpression. (**F**) Survival curve of mice receiving STAT5B WT or mutant transduced HSPCs, showing accelerated myeloid leukemia development in the STAT5B mutant group compared to the STAT5B wild-type group ($p < 0.05$), with further expedited leukemia development upon TET2 deletion ($p < 0.01$).