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LYMPHOID NEOPLASIA

Frequent somatic *TET2* mutations in chronic NK-LGL leukemia with distinct patterns of cytopenias

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KEY POINTS

- ***TET2* mutations are present in 28% of chronic NK-LGL leukemia, and TET2 promoter methylation is also detected.**
- ***TET2* and *STAT3* mutated singly or comutated are associated with distinct patterns of cytopenias, as well as response to immunosuppressives.**

Chronic natural killer large granular lymphocyte (NK-LGL) leukemia, also referred to as chronic lymphoproliferative disorder of NK cells, is a rare disorder defined by prolonged expansion of clonal NK cells. Similar prevalence of *STAT3* mutations in chronic T-LGL and NK-LGL leukemia is suggestive of common pathogenesis. We undertook whole-genome sequencing to identify mutations unique to NK-LGL leukemia. The results were analyzed to develop a resequencing panel that was applied to 58 patients. Phosphatidylinositol 3-kinase pathway gene mutations (*PIK3CD*/*PIK3AP1*) and *TNFAIP3* mutations were seen in 5% and 10% of patients, respectively. *TET2* was exceptional in that mutations were present in 16 (28%) of 58 patient samples, with evidence that *TET2* mutations can be dominant and exclusive to the NK compartment. Reduced-representation bisulfite sequencing revealed that methylation patterns were significantly altered in *TET2* mutant samples. The promoter of *TET2* and that of *PTPRD*, a negative regulator of *STAT3*, were found to be methylated in additional cohort samples, largely confined to the TET2 mutant group. Mutations in *STAT3* were observed in 19 (33%) of 58 patient samples, 7 of which had concurrent *TET2* mutations. Thrombocytopenia and resistance to immunosuppressive agents were uniquely observed in those patients with only *TET2* mutation (Games-Howell post hoc test, $P = .0074$; Fisher's exact test, $P = .00466$). Patients with *STAT3* mutation, inclusive of those with *TET2* comutation, had lower hematocrit, hemoglobin, and absolute neutrophil count compared with *STAT3* wild-type patients (Welch's *t* test, $P \leq .015$). We present the discovery of *TET2* mutations in chronic NK-LGL leukemia and evidence that it identifies a unique molecular subtype.

Introduction

Chronic natural killer large granular lymphocyte (NK-LGL) leukemia is a rare disorder defined by prolonged expansion of clonal NK cells. It is recognized by the World Health Organization 2016 classification as the provisional entity chronic lymphoproliferative disorder of NK cells.[1] The clinical presentation of persistent NK cell expansion and associated symptoms such as cytopenias are shared with chronic T-cell LGL (T-LGL) leukemia.[2,3] The seminal discovery of *STAT3* mutations in chronic T-LGL leukemia[4] and subsequent similar findings in chronic NK-LGL leukemia have suggested a common pathogenesis.[5] However, research in chronic NK-LGL leukemia has been biased in that discovery is often performed in patients having predominatly T-LGL leukemia. For example, in the largest published LGL leukemia exome study to date, encompassing 19 exomes, only 3 were of the chronic NK-LGL type.[6]

Multiple correlations have been observed between *STAT3* mutations and clinical behavior in chronic T-LGL leukemia, including the association between Tyr640Phe *STAT3* mutations and favorable response to methotrexate (MTX),[7] and the association of *STAT3* mutations with neutropenia.[8] Recently, associations between *STAT3* mutations and cytopenias have been reported in chronic NK-LGL leukemia in several studies involving small numbers of patients. These results include a statistically significant reduction in hemoglobin and a trend toward lower neutrophil count,[9] and further evidence of linkage between neutropenia and *STAT3* mutation.[10,11] Here, we report clinical correlations from a cohort that exceeds the combined patient numbers of these previous studies.

The TET2 enzyme converts the modified base 5-methylcytosine to 5-hydroxymethylcytosine, a step in the cascade that demethylates DNA. *TET2* is known to be recurrently mutated in childhood[12] and adult[13] acute myeloid leukemia (AML). *TET2* mutation is also prevalent in myelodysplastic syndrome and nearly half of all cases of the related chronic myelomonocytic leukemia.[14] Other than frank leukemia, *TET2* is heavily involved in age-related clonal outgrowth in the hematopoietic compartment.[15-19] However, in this condition, *TET2* mutation has been shown to have a distinct myeloid and B-cell proliferation bias with a complete lack of mutant-derived T-cell proliferations.[20] It is possible this bias affects the common precursor of T and NK cells, which would dictate that *TET2* mutations in these lymphoid cells are predominantly peripherally acquired. Mutations in *TET2* or other methylation pathway enzymes in the lymphoid compartment are observed in peripheral T-cell lymphoma.[21,22] In LGL leukemia, *TET2* mutation has been reported in 1 patient of an Italian NK-LGL cohort[23] and in 1 T-LGL leukemia case report.[24]

In the current study, we present results from what is currently the largest chronic NK-LGL mutation discovery cohort of 7 whole-genome sequencing (WGS) samples, with validation by targeted resequencing in 51 additional samples. We discovered that *TET2* is frequently mutated and that the mutations change the methylation landscape in this cohort, highlighting that *TET2* also has a major role in a lymphoid malignancy. *STAT3* was also highly mutated in these samples, and thus this cohort provided a unique opportunity to compare and contrast the clinical features of the 2 most common mutations presently known in chronic NK-LGL leukemia.

Methods

Patient consent and clinical records

All studies were conducted under investigational review board–approved protocols for the LGL Leukemia Registry at the University of Virginia, with informed consent. All patients met ≥1 of the following criteria for a diagnosis of NK-LGL leukemia: $>2 \times 10^9$/L atypical lymphocytes; evidence of NK-cell invasion of the marrow; and aberrant NK populations detected by using flow cytometry. Concurrent myelodysplasia was not noted in any of the 48 available marrow biopsy specimens from our cohort of 58. Patient demographic characteristics, complete blood counts (CBCs), and flow cytometry data were collected from the time point nearest to when the sequenced sample was collected to ensure accurate correlation with mutation status. Treatments received at any point and their timing relative to sequencing and diagnosis were also collected. Strict criteria were used for treatment indication and response to therapy as previously described.[7] Response was considered unevaluable if the treatment indication was not cytopenias. These data are presented together in supplemental Table 1 (available on the *Blood* Web site).

Patient sample purity assessment and enrichment

We set a target of 70% leukemic cells in each WGS sample so that we would be able to identify minor clones and subclones. Clinical flow results of NK cell markers for patients 1862, 1856, and 1791 (94% $CD94^+$, 76% $CD56^+$, and 93% $CD56^+$, respectively) indicated these samples were suitable for WGS without enrichment. Patient 1444 was enriched with a negative selection NK Cell Isolation Kit (Stemcell Technologies). Patients 1511, 1820, and 1866 were positively selected with a CD94 antibody (clone REA113) on the AutoMACS system (Miltenyi Biotec), as were samples later used for Sanger sequencing validation of *TET2* mutations. The $CD94^-$ population consisted of the remainder of the peripheral blood mononuclear cells not captured by the enrichment.

Whole-genome, targeted, and Sanger sequencing

We completed WGS of matched peripheral blood mononuclear cells (PBMCs) or enriched NK cells (described earlier) and saliva from 7 patients with NK-LGL at a target of 40× normal and 80× tumor coverage (Illumina). PBMC DNA was extracted by magnetic bead isolation (AnaPrep; BioChain Institute). Saliva DNA was prepared with Oragene OG-500 Colletion Kits (DNA Genotek). Mutect2[25] was used to identify somatic mutations in this cohort using COSMIC v77[26] and dbSnp v138 as whitelist and blacklist, respectively (supplemental Table 2). Select genes with somatic mutations in this discovery cohort, and 3 from the literature, were used to develop a comprehensive targeted resequencing panel of all exon boundaries (supplemental Table 3). This panel was used to validate the variant calls from the 7 WGS samples and sequence an additional 28 paired PBMC/saliva samples and another 23 PBMC-only samples. Libraries were prepared by using the Ion AmpliSeq Kit for Chef DL8, and reads were collected on the Ion Torrent S5 (Thermo Fisher Scientific). An average per-sample target coverage of 1200× was achieved, with the lowest being 535×. Mutations were called and annotated by using the unmodified Ion Reporter 5.6 tumor/normal workflow for 35 paired samples and tumor-only for all 58 samples. Five recurrently mutated genes found in the tumor/normal pairs were thoroughly scrutinized in the tumor-only cohort. Lack of normal DNA in the tumor-only cohort necessitated that we apply computational filters to remove variants of germline origin before analysis. We removed calls that were not found in COSMIC (Catalogue of Somatic Mutations in Cancer), had poor alignments, or had a global allele frequency >0.1% in the 1000 Genomes Project[27] version of 20161108.

The supplemental Methods present full details of Sanger sequencing and reduced-representation bisulfite sequencing (RRBS).

Statistical analysis

Statistical analyses for the clinical associations were performed by using R statistical software, version 3.5.3, with a significance level of $\alpha = 0.05$. Welch's *t* test was performed on CBC parameters for wild-type (WT) vs *STAT3* mutant comparisons, as well as WT vs *TET2* mutant comparisons. A 1-way analysis of variance (ANOVA) Welch's test was performed on CBC parameters for WT, *TET2*-only, *STAT3*-only, and comutation groups. Games-Howell post hoc tests were performed on CBC parameters significant in the ANOVA (platelets). A Fisher's exact test was used to assess if the binary ratio of parameter of interest is different for WT, *TET2*-only, *STAT3*-only, and comutation groups for non-CBC comparisons such as survival and treatment and followed by a pair-wise Fisher's exact test when significant.

Results

WGS indicates significant clonality in all NK-LGL leukemia

Initial analysis of 7 whole-genome sequences using Mutect2[25] indicated a median 52 nonsilent mutations per sample, with the

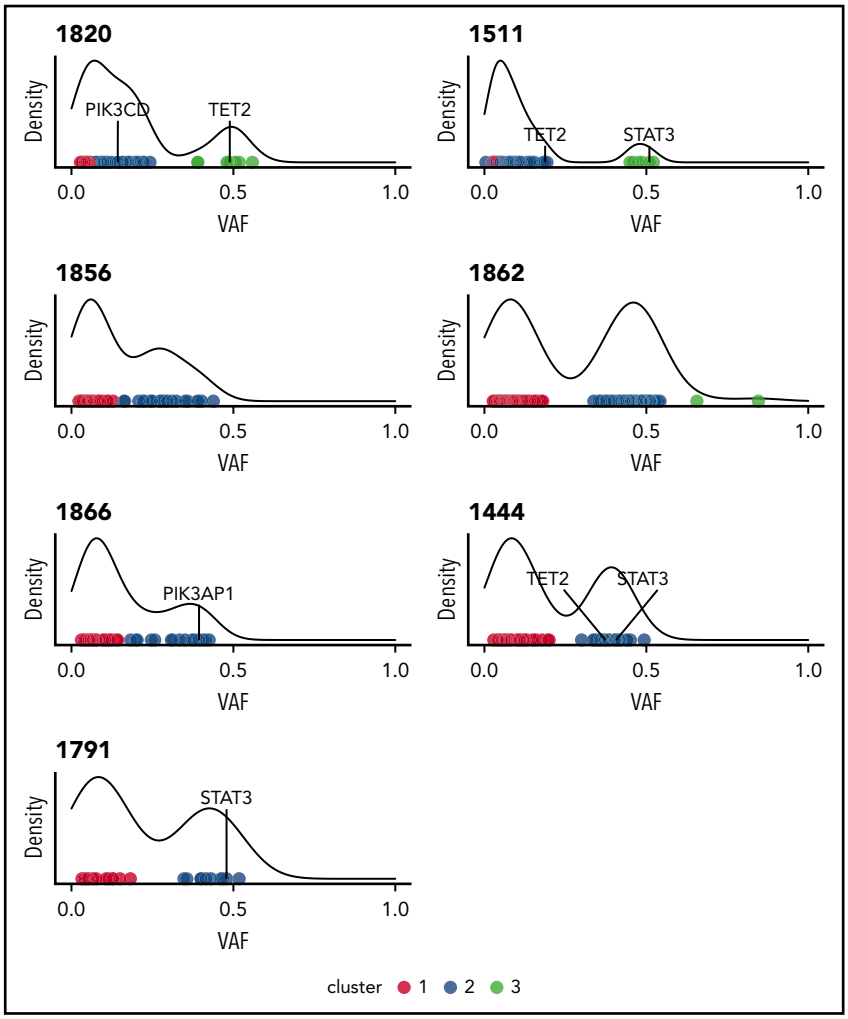

Figure 1. Clustering variants using variant allele frequencies (VAFs) of protein-altering variants reveals heterogeneity and clonality in NK-LGL leukemia. Density plots are shown for the 7 patients who underwent WGS. All indicate the presence of at least one major clone. Individual points are different somatic mutations, and color signifies cluster membership. Genes found to be recurrently mutated in this study are indicated.

somatic nonsilent mutation burden varying from 25 to 101 mutations (supplemental Table 2). Based on HMMcopy[28] analysis, 2 of the samples (1820 and 1862) exhibited evidence of large-scale somatic copy number variants (supplemental Figure 2). In most samples, the variant allele frequencies of nonsilent mutations clustered into those with high (~0.5) and low values. The former indicated the presence of a major dominant clone (Figure 1). Of the genes with nonsilent mutations, 2 potential drivers present in dominant clones (STAT3 and TET2) were identified by taking the union of genes predicted by IntOGen[29] and MutSigCV.[30]

Validation of select mutations and paired sample resequencing

The WGS calls from the discovery cohort were validated by using a targeted resequencing panel of 39 genes. Mutations in 26 of the genes, including STAT3 and TET2, were validated in targeted resequencing of paired tumor/normal samples (Figure 2). In the 7 WGS sample validations and additional 28 paired samples, nonsilent mutations in TET2 were found in 9 (26%) of 35, often at frequencies that indicated presence in the majority of NK cells (supplemental Table 1). STAT3 mutations were seen in 12 (34%) of 35 samples in the paired analysis. Two of the 28 additional paired samples contained mutations in either TNFAIP3 or PIK3CD, which implicates the NF-κB and phosphatidylinositol 3-kinase (PI3K) pathways.

We further expanded our investigation to an additional 23 samples that lacked paired normal samples. We focused only on those genes recurrently mutated in the paired analysis: STAT3, TET2, TNFAIP3, and PI3K pathway genes PIK3CD and PIK3AP1. Examination of all 58 samples revealed that 3 (5%) of the samples were mutated in the PI3K pathway genes. Both mutations noted in PIK3CD are presumed to be activating as they have been observed as de novo germline mutations causing activated PI3 kinase delta syndrome,[31] whereas the PIK3AP1 mutation has not been previously described. Twice as many (6 of 58 [10%]) samples were mutant in TNFAIP3.

Nonsilent mutation frequency for TET2, with mutations observed in 16 (28%) of all 58 samples, was consistent with that seen in paired samples alone (26%). The majority of TET2 mutations were truncations leading to loss of the catalytic domain (Figure 3A) and are rated pathogenic or likely pathogenic according to American College of Medical Genetics and Genomics/Association for Molecular Pathology standards as implemented in VarSome[32] (supplemental Table 4). Point mutations were located in regions of the catalytic domain previously established to lead to loss of TET2 function when altered.[33] Six patients had ≥2 TET2 mutations

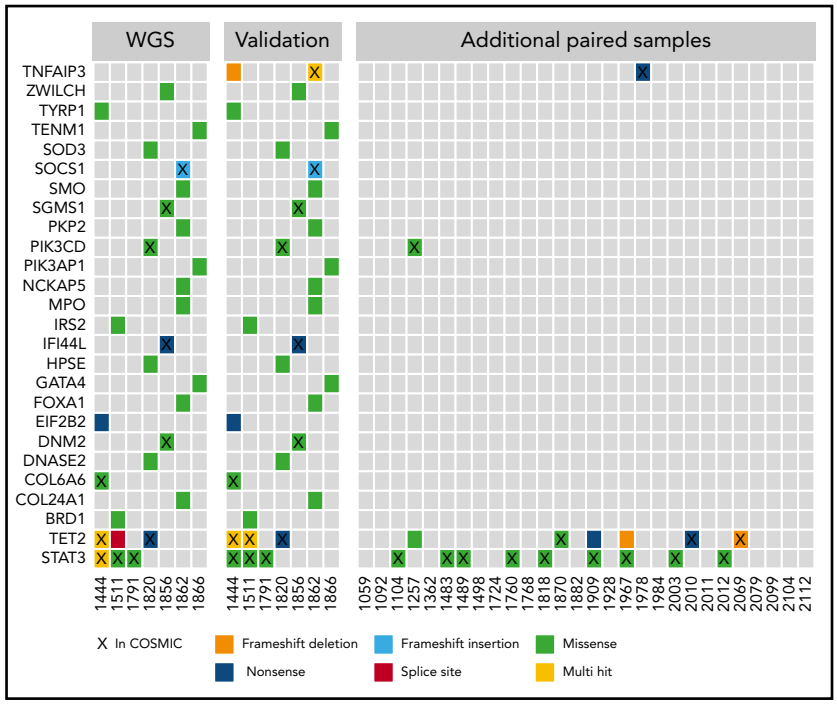

Figure 2. Select mutations found in WGS and targeted resequencing. The first panel shows the mutation distribution and type in the initial 7 whole-genome samples that were the basis for a targeted resequencing panel. The second panel shows validations on the targeted panel for the same samples and indicates good concordance between sequencing methods. The third panel shows additional paired PBMC/saliva samples sequenced by using the targeted panel, which identified few recurrently mutated genes in this cohort with the exceptions of *TET2* and *STAT3*. Genes are listed by official gene symbol and samples by registry identification number.

present in the same NK-LGL sample. Of note, we omitted the TET2 mutation Gln599Arg as it seemed to be a rare germline variant as opposed to its previously reported somatic nature in myeloid neoplasms[34] (supplemental Figure 3). We also explored alternative mechanisms of TET2 loss in our NK-LGL cohort. We observed no known defective TET2 germline variants or TET2 copy number loss in whole-genome samples. However, methylation-specific polymerase chain reaction[35] revealed TET2 promoter methylation predominantly in NK-LGLs with somatic TET2 mutation (9 of 14 [64.3%]) rather than those that were WT for TET2 (1 of 21 [4.8%]; Fisher's exact test, $P = .0002$) (Figure 3B-D; supplemental Figure 4). Of those with TET2 mutations, promoter methylation was more prevalent in those samples with single (7 of 10 [70%]) rather than compound (2 of 6 [33.3%]) TET2 mutations, which suggests this may be a mechanism to silence expression of the WT allele.

STAT3 was mutated in 19 (33%) of 58 samples, and these were predominantly missense mutations localized to the SH2 domain, which have been previously shown to increase STAT3 activity[4] (Figure 3A). Of 7 samples with mutations in both *STAT3* and *TET2*, mutation frequencies suggested 4 (1257, 1444, 1507, and 1909) to be in the same clone, whereas the frequencies differed substantially for 3 (1001, 1511, and 1967), suggesting they are present in different clonal populations. Mutation type and allele frequency for *STAT3, TET2, TNFAIP3, PIK3CD,* and *PIK3AP1* are listed in supplemental Table 1 with clinical correlates.

Additional evidence that TET2 mutations are present in the NK compartment

Samples 1444, 1511, and 1820 were enriched for NK cells before WGS and found to be TET2 mutant. We took this as preliminary evidence that mutations were indeed in the NK compartment. To validate this, NK^+ and NK^- fractions were isolated from patient 1511 and five additional patients, which achieved at least 84% $CD94^+CD8^-$ cells in the NK^+ fraction. Nine mutations in 6 samples (3 samples had 2 mutations) were then tested by Sanger sequencing of TET2 (examples are given in Figure 3E, with full results in supplemental Figure 1). All 6 patients tested by using Sanger sequencing had ≥1 TET2 mutation present only in the NK^+ cell compartment, including sample 1511, which had 2. Two patients, 1644 and 1967, had second TET2 mutations that were more pronounced in the NK^+ vs NK^- sample. One of these, Glu1268Ter in 1967, was also found in the patient's saliva at an allele frequency of 16%. This finding could indicate the presence of the mutation in neutrophils[36] and a cooccurring myeloid disorder or clonal hematopoiesis, but this patient's marrow morphology was unremarkable. No other saliva samples showed the presence of mutations in TET2, STAT3, TNFAIP3, or PI3K genes. Between next-generation and Sanger sequencing, all 8 patients with material available for enrichment exhibited ≥1 TET2 mutation solely in the NK compartment.

TET2 mutant NK-LGL leukemia displays altered global methylation

The enzyme encoded by *TET2* catalyzes a step in the demethylation of DNA; we therefore performed RRBS to ascertain the methylation changes in patient samples relative to *TET2* mutational status. Leukemic samples were a subset of those fully characterized by WGS. Principal component analysis of the data showed that samples with mutations in *TET2* exhibited significant separation from the other NK-LGL samples and normal NK-cell control samples (Figure 4A). Less separation was seen for mutant sample 1511, which may be explained by the fact that the *TET2* mutation was observed in the minor clone for this sample (Figure 1). In comparisons between leukemic samples and normal NK cells, nearly 5 times more differentially methylated regions were observed in *TET2* mutant samples (Figure 4B). This finding suggests that the mutations identified are associated with disrupted *TET2* function.

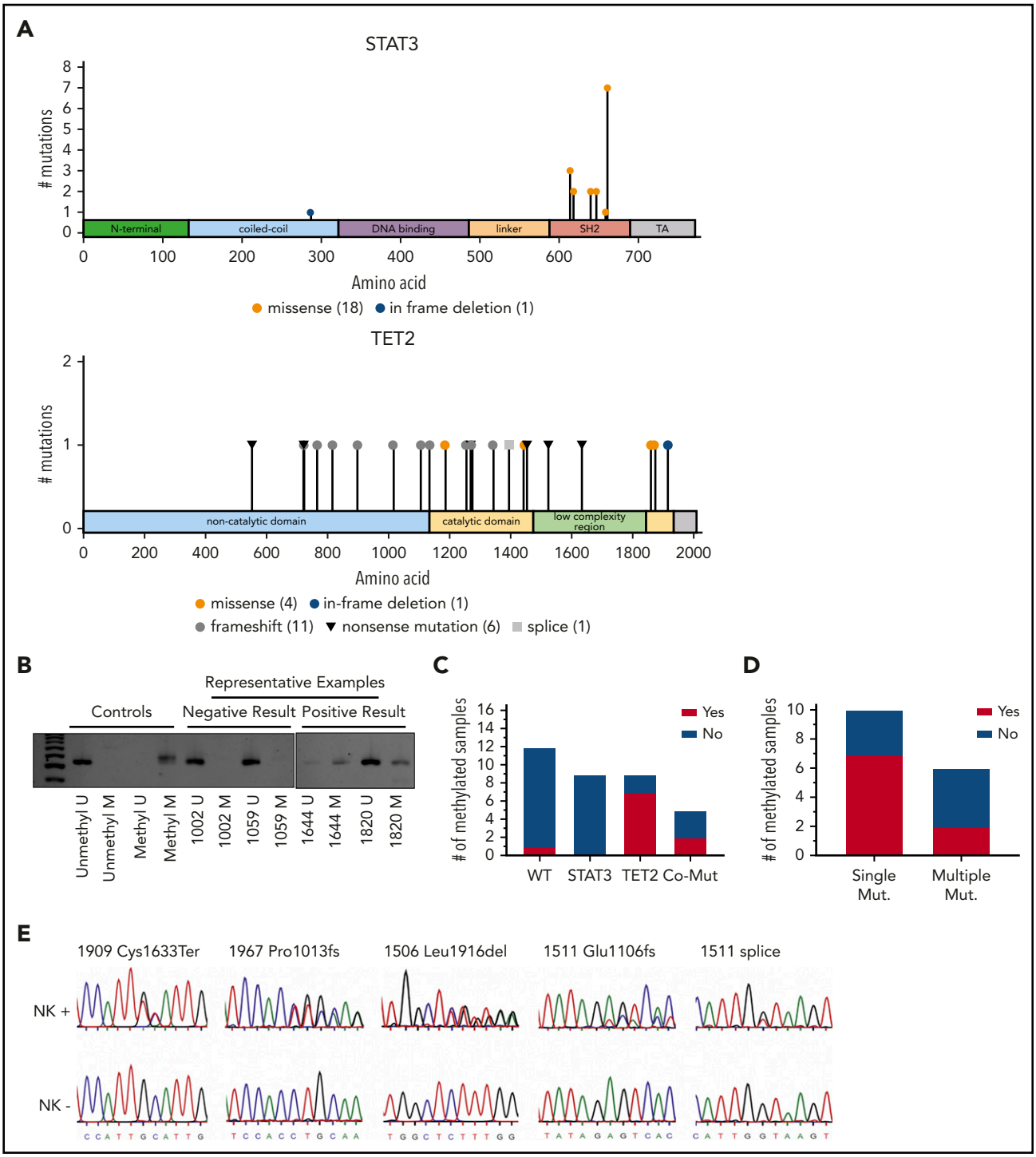

Figure 3. Spectrum of somatic mutations in STAT3 and TET2. (A) Lollipop plots indicate the frequency (y-axis) and placement within the amino acid sequence (x-axis) of detected mutations in STAT3 and TET2 proteins. (B-D) TET2 promoter methylation–specific polymerase chain reaction assay. Representative negative and positive patient sample results are indicated (B), and all gels are provided in supplemental Figure 4. (C) TET2 promoter methylation within mutational subgroups. (Fisher's exact test, TET2 WT vs TET2 Mutant [Mut] [all samples], $P = .0002$; WT vs TET2 Mut, $P = .0022$). (D) TET2 promoter methylation of all TET2 Mut samples divided into those with ≥1 mutation. Fisher's exact test, not significant, $P = .3024$). (E) Representative Sanger sequencing from isolated CD94$^+$ and CD94$^-$ fractions showing that TET2 mutations are exclusively detected in the C94$^+$ NK fraction. Co-Mut, comutation.

We next annotated differentially methylated regions to gene promoters (full data set in supplemental Table 5). Pathway analysis revealed hypermethylation of hundreds of transcriptional activators and regulatory region–binding factors associated with RNA polymerase II (supplemental Tables 6 and 7), suggesting the potential for aberrant epigenetic regulation of many downstream targets. We hypothesized that *TET2* mutation may contribute to NK-LGL leukemia by leading to loss of negative regulators of *STAT3*. We therefore analyzed the promoter regions of putative negative regulators (PIAS, PTP, PTPR, SHIP, and SOCS gene

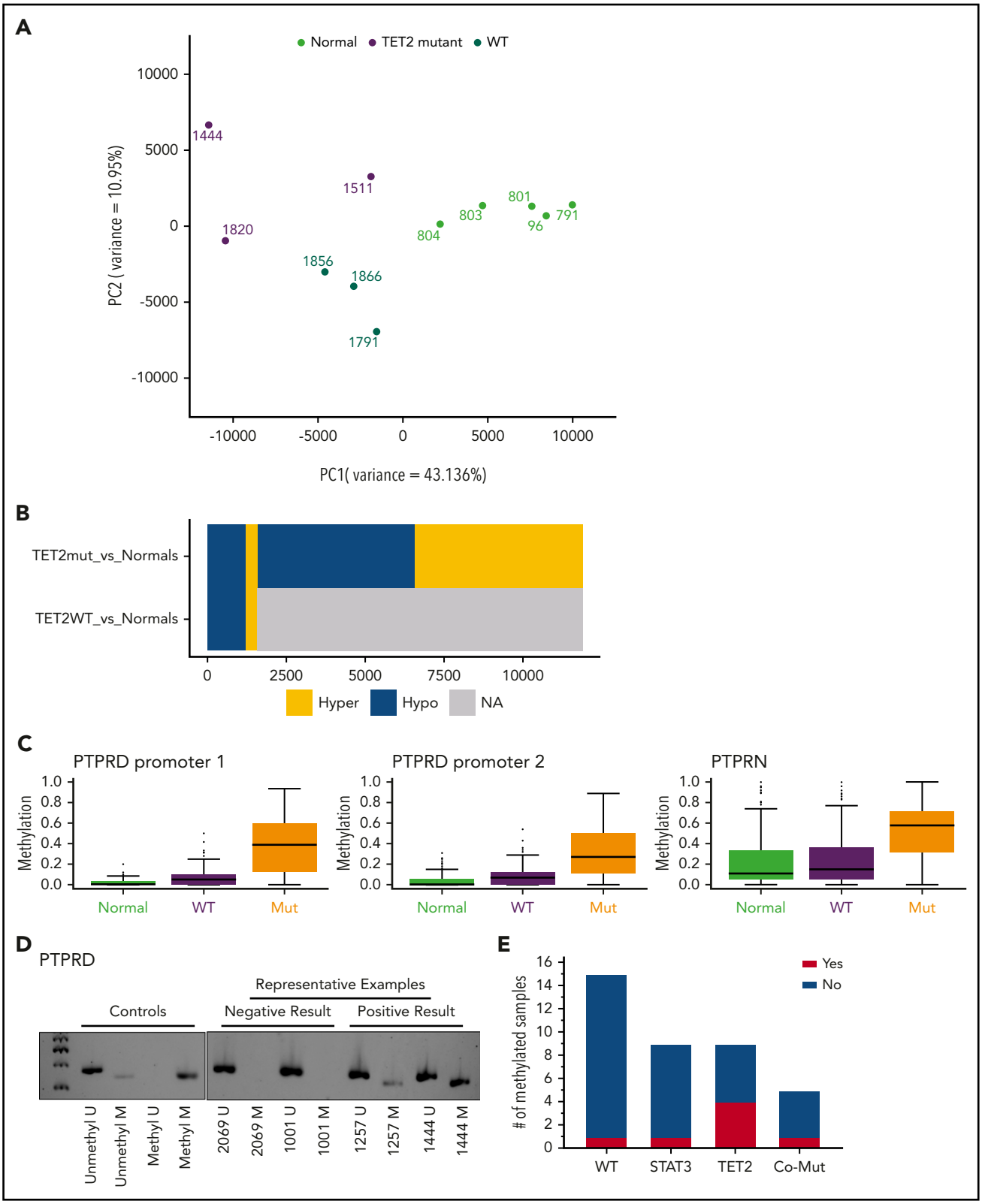

Figure 4. RRBS assessment of changes in DNA methylation in NK-LGL patient samples compared with normal NK cells. (A) RRBS was used to assess DNA methylation in the same highly pure leukemic samples used for WGS (TET2 mutant [Mut], n = 3; TET2 WT, n = 3) compared with 5 purified NK cell samples from normal donors (Normal). Principal component analysis used high-variance cytosine-phosphate-guanine sites at the 0.9 percentile of the interquartile range. Number of data points = 239 696. (B) Comparison of number of hypermethylated (Hyper) and hypomethylated (Hypo) differentially methylated regions of TET2 Mut and TET WT leukemic samples vs normal NK cells. (C) Distribution and quartiles of the amount of individual cytosine methylation within promoters of negative regulators of STAT3 in which 1 = 100% methylated. (D) Representative PTRD promoter methylation–specific polymerase chain reaction, with all gels provided in supplemental Figure 5. (E) PTPRD promoter methylation within mutation subgroups (Fisher's exact test, TET2 WT vs TET2 Mut [all samples] not significant, $P = .0772$; WT vs TET2 Mut, $P = .0474$. Co-Mut, comutation; NA, not applicable; PC, principal component.

families) and found that those in protein tyrosine phosphatase receptor type delta (PTPRD) and type N were the most differentially methylated in TET2 mutant leukemic samples (Figure 4C; supplemental Figure 5) compared with TET2 WT LGL and normal NK control samples. Loss of PTPRD has been shown to lead to aberrant STAT3 activation.[37-39] We therefore expanded the assessment of PTPRD promoter methylation using methylation-specific polymerase chain reaction[39] on samples from our cohort; representative results are given in Figure 4D and full results in supplemental Figure 6. We found that 44.4% (4 of 9) of TET2-mutated samples exhibited promoter methylation (Figure 4E). This compares to 6.7% WT (1 of 15), 11.1% STAT3 mutant (1 of 9), and 25% comutant (1 of 5). This scenario suggests an alternative mechanism of STAT3 activation in TET2 mutant samples.

TET2 mutations are persistent in untreated NK-LGL leukemia

We next sequenced longitudinal tumor samples from 4 patients with TET2 mutations who did not receive treatment with cyclophosphamide (CP), MTX, or cyclosporine (Figure 5A), with a focus on the 5 recurrently mutated genes. Three patients had TET2 mutations at the beginning of observation, and patient 1511 developed a TET2 mutation at 70 months. TET2 mutations persisted indefinitely after detection in these untreated samples over 36, 40, 61, and 155 months, with an average of 73 months' follow-up. TET2 mutation followed STAT3 mutation in patient 1511; the opposite was observed for patient 1257, in which TET2 mutation was followed by STAT3 mutation. In both patients, the frequencies for the 2 mutations appeared to converge, which may indicate presence in the same clone. Taken together, these data show that TET2 inactivation is associated with persistent clonal expansions with and without STAT3 mutation.

Variant allele frequencies correlate with response to CP

Four patients found to be TET2 mutant in this study underwent treatment with CP (100 mg daily orally) and had pretreatment and multiple posttreatment samples available. Clinical records for all 4 patients indicated complete response to therapy (ie, an improvement in blood counts). Samples from each of the 4 patients were then sequenced with the targeted panel, and mutational profiles were compared with disease burden as assessed by absolute lymphocyte count (Figure 5B). Of these, patients 1507 and 1909 showed elimination of the TET2 mutant clone with no return in the 117- and 40-month follow-up period for each sample, respectively. Patient 1444 showed initial elimination of the TET2 mutant clone, which re-emerged but not until 49 months later and in tandem with the emergence of a TNFAIP3 mutation. All 3 of these long-term responding patients had STAT3 mutations of frequency similar to that observed for TET2. Patient 1967 was unique in that 1 TET2 mutation (Pro1013fs) we had previously determined to be in the NK compartment was reduced by treatment, whereas the other (Glu1268Ter) was not greatly reduced. In all treated samples, the reduction in variant allele frequencies was accompanied by concomitant reduction in the lymphocyte count.

Clinical associations

We analyzed the effect of STAT3 mutation and TET2 mutation independently on CBC parameters to determine their possible correlation with cytopenias known to be present in patients with LGL leukemia. Highly significant ($P < .02$) decreases were observed in absolute neutrophil count, hemoglobin, hematocrit, and red blood cell (RBC) counts and increased RBC distribution width in STAT3 mutant samples compared with those without (Figure 6). Although similar trends are noted for TET2 mutants, none of the differences reached statistical significance. All tested CBC comparisons, including nonsignificant findings, are presented in supplemental Figures 7 and 8. These analyses were completed for CBC data taken on or near the date of the sequenced sample to ensure that the mutations were present. Some patients were undergoing treatment at that time. However, similar trends were observed in comparisons of CBCs from an additional treatment-naive date (supplemental Figure 9).

We then determined whether single mutations in either TET2 or STAT3 as opposed to mutations in both or neither lead to significant changes in CBC parameters. Overall, there was a pattern of reduced RBC, hemoglobin, hematocrit, and absolute neutrophil counts according to mutation profile. Slight decreases were observed in TET2-only mutants and modest decreases in STAT3-only mutants; the largest decreases were observed in comutated patients (supplemental Figure 5). Platelet count was the only parameter to show statistically significant global change (1-way ANOVA, $P = .0071$) (Figure 7A). Pairwise comparison revealed that the TET2-only mutation group had a significantly reduced platelet count compared with the WT group ($P = .0074$). Figure 7B shows that a number of these patients met the clinical definition of thrombocytopenia (<150 000/μL) and exhibited higher mean platelet volume than other groups.

We also examined other categorical clinical variables relative to STAT3 and TET2 mutational status. Overall survival to 60 months for all mutation groups was ≥80%, and differences according to mutation status have not emerged at this point (Figure 7C). TET2 mutation, alone or in combination with STAT3, was associated with a slightly increased need for immunosuppressive treatment by 60 months that failed to reach statistical significance (Figure 7D). Roughly half (15 of 32 patients) of patients in the entire cohort who sought immunosuppressive treatment achieved at least some response to either MTX or CP (supplemental Table 1D).

We then examined treatment response between the groups as response (complete + partial) vs no response to MTX or CP. The patients with TET2 mutation exhibited significantly reduced response rates to MTX or CP treatment compared with patients with a STAT3 single mutation (pairwise Fisher's exact test, $P = .00466$) (Figure 7E). All 4 TET2/STAT3 comutation patients exhibited a complete response to CP, which is in sharp contrast to the single complete response to CP observed in 5 patients with TET2 single mutation. The sex ratio was significantly different between the WT and TET2/STAT3 comutation group, with the TET2/STAT3 comutation group reporting no female patients (pairwise Fisher's exact test, $P = .0279$) (Figure 7F).

Discussion

The key finding from the current study is that targeted sequencing of select mutations from a discovery cohort of 7 WGS samples found that *TET2* was mutated in 28% of chronic NK-LGL leukemia cases. This frequency approaches that of previously reported *STAT3* mutations, which we found in 33% of samples. Unlike *STAT3* mutations, *TET2* mutations seem to be specific to the

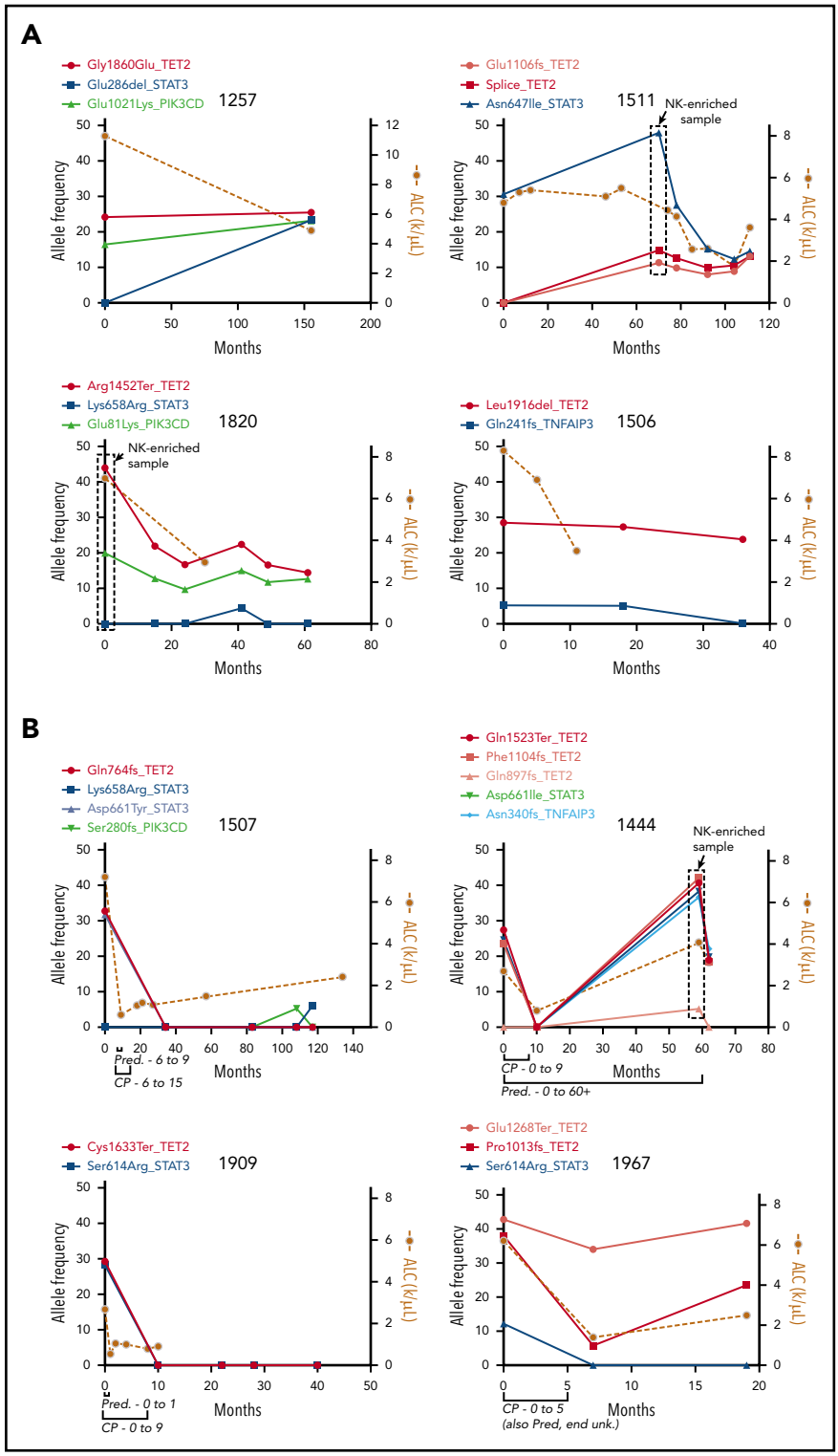

Figure 5. Longitudinal measurement of mutation allele frequency in *TET2* mutant PBMC samples. Variant allele frequencies (left y-axis) are shown in the 5 recurrently mutated genes as measured by targeted sequencing and plotted with leukemic burden as assessed by absolute lymphocyte count (ALC; right y-axis). (A) Four untreated samples showing long-term persistence of *TET2* mutant clones. The boxed data points are from NK-enriched samples, and thus allele frequencies are skewed higher. (B) Four samples that received treatment with CP for 9 or 5 months as indicated, showing complete reduction of the mutant clone and lymphocyte counts in treated individuals. Pred, prednisone; unk., unknown.

NK variant of LGL leukemia as they have not been reported in T-LGL leukemia thus far beyond 1 case.[24] We showed that all testable patients had a mutation solely in the NK compartment. However, one should be cognizant that LGL leukemia could coincide with other disorders, which could also be *TET2* mutant. We established that TET2 mutations are clearly in the NK compartment, but we cannot rule out their existence in precursors and other cell types as we interrogated $CD94^{+}$ and $CD94^{-}$ fractions via Sanger sequencing. It remains a formal possibility that deeper next-generation sequencing analysis may have picked up low-level mutations in the nonleukemic $CD94^{-}$ compartment.

Our preliminary analysis of CP-treated patients with NK-LGL leukemia indicates that *TET2*-specific treatments may not be

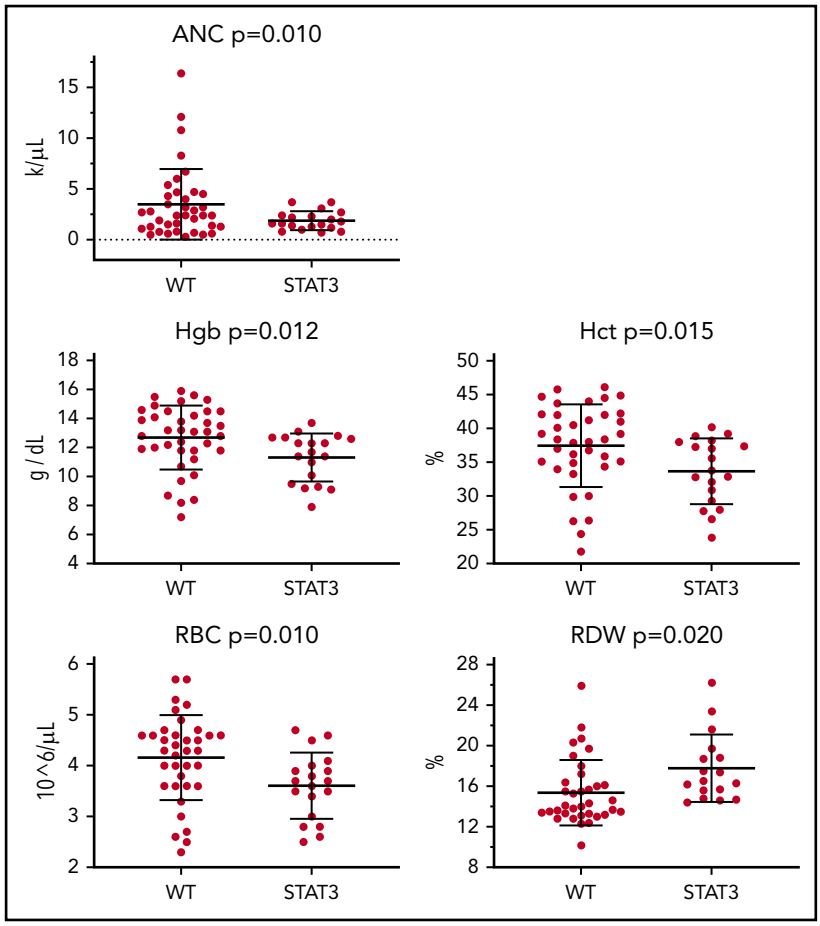

Figure 6. CBC parameters for patients with *STAT3* mutation. CBC values from the date closest to the sequenced date are stratified according to patients who are *STAT3* mutant (n = 20) or non–*STAT3* mutant (WT) (n = 38). ANC, absolute neutrophil count; Hct, hematocrit; Hgb, hemoglobin; RDW, RBC distribution width.

necessary to achieve clinical response in *TET2/STAT3* comutant samples. However, our observation of methylation differences in all leukemic samples, compared with normal NK cells, would suggest methylation as a potential therapeutic target in all patients with NK-LGL leukemia. The decision to use the hypomethylating agent azacytidine in AML is often driven by patient age and other risk factors, and not by the presence or absence of mutant TET2.[40,41] Those patients with NK-LGL leukemia with only *TET2* mutations stand to gain the most from new therapies, as only 1 of 5 followed up in this study responded to either CP or MTX. The finding of TET2 promoter methylation in TET2 mutant samples suggests these mechanisms cooperate to fully abrogate TET2 function. This action may make this subgroup susceptible to inhibition of other TET family members as at least some residual activity is required for clonal outgrowth.[42] Unfortunately, models that introduce *TET2* dysfunction into the marrow do not recreate NK-LGL leukemia; thus, genetically accurate preclinical models to screen and optimize experimental therapeutics are lacking.[43] In a model similar to LGL leukemia but benefiting from defined stimulation, *TET2* disruption also led to robust proliferation and expansion of chimeric antigen receptor T cells.[44] Models of *TET2* mutation in myeloid disorders[45,46] have shown a requirement for additional cooperative mutations to promote leukemic expansion, and we speculate that this is also true for NK-LGL leukemia.

Our strategy of targeted resequencing identified *TET2* and a few other genes as being mutated. *TNFAIP3* and *PI3K* pathway genes exhibited mutations in 10% and 5% of this cohort, respectively. *TNFAIP3* mutation has been previously described in chronic T-LGL[47] and NK-LGL[9] leukemia. *PIK3AP1* and *PIK3CD* mutations have not previously been reported in LGL leukemia, and it is noteworthy that clinical trials of compounds targeting this pathway are advancing in other cancers.[48]

In the WGS samples of this study, in accordance with past exome sequencing, all samples showed a significantly mutated clone. This finding suggests that the phrase "chronic lymphoproliferative disorder of NK cells" is a misnomer that implies these are not highly clonal expansions. There is an obvious need to distinguish chronic and aggressive NK leukemias, but the term chronic NK-LGL leukemia may more accurately represent the similarities between this and the chronic T-LGL form. It will be interesting to observe, in other patient populations, how many samples will prove to be clonal. We can now theoretically measure clonality in roughly half of chronic NK-LGL by sequencing *TET2* and *STAT3* alone, which may facilitate this analysis in the future. We would expect more oligoclonal proliferation in earlier disease. This is consistent with the belief that antigen is the true driver of LGL leukemia[49] and that mutations are opportunistic clonal expanders.[50]

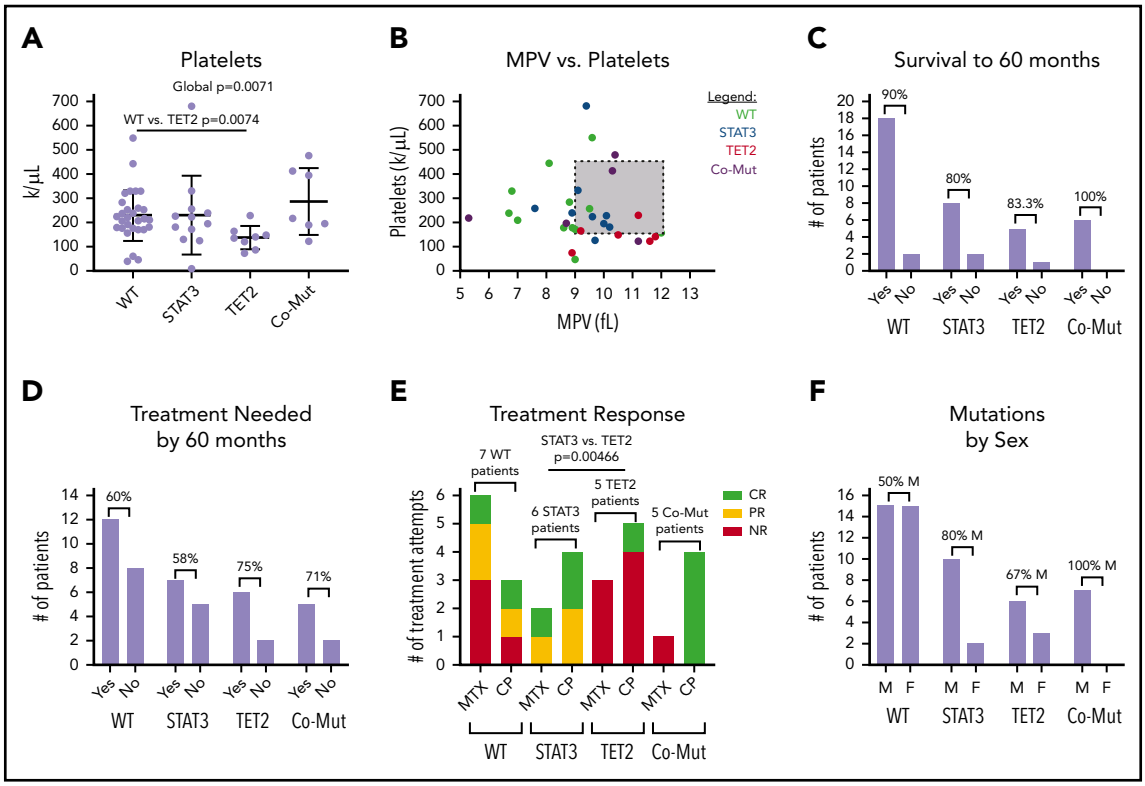

Figure 7. CBC parameters and other clinical features stratified according to *STAT3* and *TET2* mutation. Patients are divided into 4 categories, nonmutant (WT) (n = 29), *STAT3* mutant-only (n = 12), *TET2* mutant-only (n = 9), and comutation (Co-Mut) of both *STAT3* and *TET2* (n = 8). Platelets (Welch's 1-way ANOVA global, $P = .0071$; Games-Howell post hoc test, WT vs TET2, $P = .0074$) (A) and relationship between platelet count and mean platelet volume (MPV) (B) in CBC reports from the date closest to the sequencing sample. (C) Overall survival within each group for those patients who have achieved 60 months of follow-up. (D) Need for immunosuppressive treatment within each group for those patients who have achieved 60 months of follow-up. (E) Response to immunosuppressive treatment initiated at any point during disease course (STAT3 vs TET2, Fisher's exact test, $P = .0046$). (F) Distribution of patient sex within mutation groups. CR, complete response; F, female; M, male; NR, no response; PR, partial response.

TET2 mutations were found in 11 samples without *STAT3* mutation, which implies they can fulfill a similar role for clonal expansion. *STAT3* is a known regulator of inflammatory cytokine production in LGL leukemia[51] in addition to its role in promoting clonal outgrowth.[50,52] This additional function may explain why *STAT3* mutant patients are more symptomatic compared with patients who are *TET2* mutant alone. It could be inferred that comutated samples, which are associated with the most severe symptoms, may represent patients who have had more long-standing disease. Thus, one could speculate that length of disease is what truly associates with clinical symptoms. Counter to that, clear differences were observed between *TET2* and *STAT3* single-mutant samples, which we would infer to be at the same stage. This finding suggests that disease duration is not, or at least not completely, responsible for symptom presentation.

Thrombocytopenia alone seems to be significantly associated with *TET2* mutant samples without *STAT3* comutation. It has been previously reported that patients with AML and *TET2* mutations have lower platelet numbers due to disruption of platelet production.[53] Our findings of low platelet counts and high mean platelet volume would suggest that platelet loss in NK-LGL leukemia takes place in the periphery. More work needs to be done to determine how this action occurs in *TET2* mutant samples and why it is specific to those without *STAT3* comutation.

We have identified altered methylation in *TET2* mutant NK-LGL samples. These changes are far more pronounced compared with those that distinguish NK-LGL leukemia from normal NK cells. *TET2* promoter methylation was largely restricted to those samples with mutation. Taken together, this may indicate that *TET2* dysregulation defines a subset of NK-LGL leukemia and is not a common feature. Roughly half of the *TET2* mutant samples (44.4%) showed methylation of the *PTPRD* promoter, a known negative regulator of *STAT3*,[37-39] which may be an alternative means of *STAT3* activation in these samples. *STAT3* mutations are abundant but not universal in the LGL leukemia patient population,[4] whereas all patients with LGL leukemia exhibit constitutive activation of STATs.[54] Damaging mutations in PTP family members have been observed in T-LGL[55] and aggressive NK-LGL[56] leukemias. Suppressor of cytokine signaling 3 (SOCS3) seems to be lost through an unknown mechanism.[57]

In summary, we identified mutant *TET2* as a major new target in chronic NK-LGL leukemia, in the largest molecular profiling study of this disease to date. We further showed its intriguing association with thrombocytopenia and resistance to CP and MTX when present without *STAT3* comutation. Although treatment indications and response to therapy were evaluated rigorously, these interesting findings should be considered preliminary until validated in prospective studies. This work improves our understanding of the heterogeneous clinical presentation of LGL leukemia.

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Authorship

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Footnotes

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There is a *Blood* Commentary on this article in this issue.

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REFERENCES

1. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.
2. Loughran TP Jr. Clonal diseases of large granular lymphocytes. *Blood*. 1993;82(1):1-14.
3. Lamy T, Loughran TP Jr. Clinical features of large granular lymphocyte leukemia. *Semin Hematol*. 2003;40(3):185-195.
4. Koskela HLM, Eldfors S, Ellonen P, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med*. 2012;366(20):1905-1913.
5. Jerez A, Clemente MJ, Makishima H, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. *Blood*. 2012;120(15):3048-3057.
6. Coppe A, Andersson EI, Binatti A, et al. Genomic landscape characterization of large granular lymphocyte leukemia with a systems genetics approach. *Leukemia*. 2017;31(5):1243-1246.
7. Loughran TP Jr., Zickl L, Olson TL, et al. Immunosuppressive therapy of LGL leukemia: prospective multicenter phase II study by the Eastern Cooperative Oncology Group (E5998). *Leukemia*. 2015;29(4):886-894.
8. Teramo A, Barilà G, Calabretto G, et al. *STAT3* mutation impacts biological and clinical features of T-LGL leukemia. *Oncotarget*. 2017;8(37):61876-61889.
9. Kawakami T, Sekiguchi N, Kobayashi J, et al. STAT3 mutations in natural killer cells are associated with cytopenia in patients with chronic lymphoproliferative disorder of natural killer cells. *Int J Hematol*. 2019;109(5):563-571.
10. Barilà G, Teramo A, Calabretto G, et al. Dominant cytotoxic NK cell subset within CLPD-NK patients identifies a more aggressive NK cell proliferation. *Blood Cancer J*. 2018;8(6):51.
11. Kurt H, Jorgensen JL, Amin HM, et al. Chronic lymphoproliferative disorder of NK-cells: a single-institution review with emphasis on relative utility of multimodality diagnostic tools. *Eur J Haematol*. 2018;100(5):444-454.
12. Langemeijer SMC, Jansen JH, Hooijer J, et al. TET2 mutations in childhood leukemia. *Leukemia*. 2011;25(1):189-192.
13. Ley TJ, Miller C, Ding L, et al; Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074.
14. Smith AE, Mohamedali AM, Kulasekararaj A, et al. Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. *Blood*. 2010;116(19):3923-3932.
15. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
16. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014;20(12):1472-1478.
17. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
18. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012;44(11):1179-1181.
19. McKerrell T, Park N, Moreno T, et al; Understanding Society Scientific Group. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep*. 2015;10(8):1239-1245.
20. Buscarlet M, Provost S, Zada YF, et al. Lineage restriction analyses in CHIP indicate myeloid bias for *TET2* and multipotent stem cell origin for *DNMT3A*. *Blood*. 2018;132(3):277-280.
21. Maura F, Agnelli L, Leongamornlert D, et al. Integration of transcriptional and mutational data simplifies the stratification of peripheral T-cell lymphoma. *Am J Hematol*. 2019;94(6):628-634.
22. Schatz JH, Horwitz SM, Teruya-Feldstein J, et al. Targeted mutational profiling of peripheral T-cell lymphoma not otherwise specified highlights new mechanisms in a heterogeneous pathogenesis. *Leukemia*. 2015;29(1):237-241.
23. Gasparini VR, Binatti A, Coppe A, et al. A high definition picture of somatic mutations in chronic lymphoproliferative disorder of

natural killer cells. *Blood Cancer J*. 2020; 10(4):42.

24. Raess PW, Cascio MJ, Fan G, et al. Concurrent STAT3, DNMT3A, and TET2 mutations in T-LGL leukemia with molecularly distinct clonal hematopoiesis of indeterminate potential. *Am J Hematol*. 2017;92(1):E6-E8.

25. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31(3): 213-219.

26. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res*. 2019;47(D1):D941-D947.

27. Auton A, Brooks LD, et al; 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015; 526(7571):68-74.

28. Ha G, Roth A, Lai D, et al. Integrative analysis of genome-wide loss of heterozygosity and monoallelic expression at nucleotide resolution reveals disrupted pathways in triple-negative breast cancer. *Genome Res*. 2012; 22(10):1995-2007.

29. Gonzalez-Perez A, Perez-Llamas C, Deu-Pons J, et al. IntOGen-mutations identifies cancer drivers across tumor types. *Nat Methods*. 2013;10(11):1081-1082.

30. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*. 2013;499(7457):214-218.

31. Michalovich D, Nejentsev S. Activated PI3 kinase delta syndrome: from genetics to therapy. *Front Immunol*. 2018;9:369.

32. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. *Bioinformatics*. 2019;35(11):1978-1980.

33. Ko M, Huang Y, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010;468(7325):839-843.

34. Ha JS, Jeon DS, Kim JR, Ryoo NH, Suh JS. Analysis of the Ten-Eleven Translocation 2 (TET2) gene mutation in myeloproliferative neoplasms. *Ann Clin Lab Sci*. 2014;44(2):173-179.

35. Chim CS, Wan TS, Fung TK, Wong KF. Methylation of TET2, CBL and CEBPA in Ph-negative myeloproliferative neoplasms. *J Clin Pathol*. 2010;63(10):942-946.

36. Barnholt KE, Hinds DA, Kiefer AK, et al. Estimation of JAK2 V617F prevalence by detection of the mutation in saliva samples from online MPN and general population cohorts. *Blood*. 2012;120(21):1737.

37. Peyser ND, Du Y, Li H, et al. Loss-of-function PTPRD mutations lead to increased STAT3 activation and sensitivity to STAT3 inhibition in head and neck cancer. *PLoS One*. 2015; 10(8):e0135750.

38. Ortiz B, Fabius AWM, Wu WH, et al. Loss of the tyrosine phosphatase PTPRD leads to aberrant STAT3 activation and promotes gliomagenesis. *Proc Natl Acad Sci USA*. 2014; 111(22):8149-8154.

39. Wang D, Wang L, Zhou J, et al. Reduced expression of PTPRD correlates with poor prognosis in gastric adenocarcinoma. *PLoS One*. 2014;9(11):e113754.

40. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood*. 2015;126(3):291-299.

41. Huls G, Chitu DA, Havelange V, et al; Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON). Azacitidine maintenance after intensive chemotherapy improves DFS in older AML patients. *Blood*. 2019;133(13): 1457-1464.

42. Guan Y, Tiwari AD, Phillips JG, Hasipek M. A therapeutic strategy for preferential targeting of TET2 mutant and TET-dioxygenase deficient cells in myeloid neoplasms. *Blood Cancer Discov*. 2020;2(2):146-161.

43. Li Z, Cai X, Cai C-L, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood*. 2011;118(17): 4509-4518.

44. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature*. 2018;558(7709):307-312.

45. Kunimoto H, Meydan C, Nazir A, et al. Cooperative epigenetic remodeling by TET2 loss and NRAS mutation drives myeloid transformation and MEK inhibitor sensitivity. *Cancer Cell*. 2018;33(1):44-59.e8.

46. Shih AH, Jiang Y, Meydan C, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell*. 2015;27(4):502-515.

47. Johansson P, Bergmann A, Rahmann S, et al. Recurrent alterations of TNFAIP3 (A20) in T-cell large granular lymphocytic leukemia. *Int J Cancer*. 2016;138(1):121-124.

48. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol Cancer*. 2019; 18(1):26.

49. Loughran TP Jr., Hadlock KG, Perzova R, et al. Epitope mapping of HTLV envelope seroreactivity in LGL leukaemia. *Br J Haematol*. 1998;101(2):318-324.

50. Kerr CM, Clemente MJ, Chomczynski PW, et al. Subclonal STAT3 mutations solidify clonal dominance. *Blood Adv*. 2019;3(6): 917-921.

51. Olson KC, Kulling PM, Olson TL, et al. Vitamin D decreases STAT phosphorylation and inflammatory cytokine output in T-LGL leukemia. *Cancer Biol Ther*. 2017;18(5):290-303.

52. Rajala HLM, Olson T, Clemente MJ, et al. The analysis of clonal diversity and therapy responses using STAT3 mutations as a molecular marker in large granular lymphocytic leukemia. *Haematologica*. 2015; 100(1):91-99.

53. Chou WC, Chou SC, Liu CY, et al. TET2 mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. *Blood*. 2011; 118(14):3803-3810.

54. Epling-Burnette PK, Liu JH, Catlett-Falcone R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest*. 2001;107(3):351-362.

55. Andersson EI, Rajala HL, Eldfors S, et al. Novel somatic mutations in large granular lymphocytic leukemia affecting the STAT-pathway and T-cell activation. *Blood Cancer J*. 2013;3(12):e168.

56. Dufva O, Kankainen M, Kelkka T, et al. Aggressive natural killer-cell leukemia mutational landscape and drug profiling highlight JAK-STAT signaling as therapeutic target. *Nat Commun*. 2018;9(1):1567.

57. Teramo A, Gattazzo C, Passeri F, et al. Intrinsic and extrinsic mechanisms contribute to maintain the JAK/STAT pathway aberrantly activated in T-type large granular lymphocyte leukemia. *Blood*. 2013;121(19):3843-3854, S1.