Published in final edited form as:

Leuk Lymphoma. 2016 May ; 57(5): 1054–1059. doi:10.3109/10428194.2015.1092527.

Chronic lymphocytic leukemia and myeloproliferative neoplasms concurrently diagnosed: clinical and biological characteristics

Gabriele Todisco, MD¹, Taghi Manshouri, PhD¹, Srdan Verstovsek, MD, PhD¹, Lucia Masarova, MD¹, Sherry A. Pierce, RN¹, Michael J. Keating, MBBS¹, and Zeev Estrov, MD¹

¹Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Chronic lymphocytic leukemia (CLL) and myeloproliferative neoplasms (MPN) may occur concomitantly. However, little is known about the pathobiological characteristics and interaction between the neoplastic clones in these rare cases of coinciding malignancies. We retrospectively examined the clinical and biological characteristics of 13 patients with concomitant CLL and MPN – 8 primary myelofibrosis (PMF), 3 essential thrombocythosis (ET), and 2 polycythemia vera (PV) – who presented to our institution between 1998 and 2014, and tested all patients for MPN specific aberrations, such as *JAK2*, *MPL* and *CALR* mutations. Along with epidemiological and molecular characterization of this rare condition, we found that *JAK2* mutation can be detected 9 years prior to PMF diagnosis, suggesting that PMF clinical phenotype may require several years to develop and CLL/MPN clinical co-occurrence might be sustained by common molecular events. Some features of these patients suggest that pathobiologies of these diseases might be intertwined.

Keywords

CLL; MPN; *JAK2*; *CALR*; *MPL*; PMF

INTRODUCTION

Chronic lymphocytic leukemia (CLL) and myeloproliferative neoplasms (MPN) are uncommon malignant disorders [1-3] that very rarely occur concomitantly [4-8]. Therapy-related MPN in patients with various neoplasms, including CLL, has been well documented [9]. However, MPN in treatment-naïve patients with CLL and CLL in patients with MPN have been reported but are poorly characterized [4-8], and the pathobiology and/or mechanism(s) responsible for their development in the same patient have not been elucidated.

Because most clinical trials and prognostic models exclude patients with unrelated neoplasms [10], the incidence of concomitant CLL and MPN is underreported and poorly studied. A recent study suggested that patients with MPN have a 2.8-fold greater risk of developing a lymphoid neoplasm than the general population [11]. Nevertheless, the

coexistence of CLL and MPN is thought to be unrelated because these diseases likely arise from different hematopoietic lineages [12].

Recent studies identified specific somatic mutations in MPN [13-14] and CLL [15-16], but the molecular abnormalities of the neoplastic cells from patients carrying both malignancies are unknown. The few published reports on concomitant MPN and CLL (or MPN and monoclonal B-lymphocytosis) provide scanty and contradictory data on the clinical characteristics of these patients and the incidence of mutations of Janus kinase (*JAK*)-2 receptor [4,6-8] and calreticulin (*CALR*) [5]. However, the prevalence of these somatic mutations in MPN with concomitant CLL has not been definitely delineated. To evaluate molecular aberrations underlying the CLL/MPN co-occurrence and define the incidence of this rare disease combination, we retrospectively reviewed a series of patients from our institution and looked for MPN specific molecular aberrations by testing each patient for *JAK2*, *MPL* and *CALR* mutations. Herein we report on the clinical and biological characteristic of 13 patients with concurrent CLL and MPN.

MATERIALS AND METHODS

Patients

After obtaining Institutional Review Board approval, we conducted a retrospective electronic chart review of 1719 patients with MPN who were seen at the leukemia clinic of The University of Texas MD Anderson Cancer Center between 1998 and 2014. Of the 1719 MPN patients, 13 had concomitant CLL. The patients' MPN and CLL diagnostic criteria were updated according to the current World Health Organization classification of hematopoietic tumors [17]. Patients with polycythemia vera (PV), essential thrombocytosis (ET), or primary myelofibrosis (PMF) were included, whereas patients with hypereosinophilic syndrome, chronic myelogenous leukemia, or B-cell lymphocytosis who did not fulfill diagnostic criteria of CLL [18] were excluded from this analysis. Medical history of every patient included in this study was extensively reviewed. Clinical characteristics including demographic data, family history, date of MPN and CLL diagnosis, Rai stage, disease-related symptoms, cytogenetic abnormalities, treatments, and survival were included in the statistical analysis. DNA of bone marrow mononuclear cells was stored at time of diagnosis or first referral, and used for *JAK2*, *CALR* and *MPL* genotyping for the purpose of this study. Informed consent was obtained from all patients in whom genetic analysis was performed. Molecular characterization of *JAK2*, *MPL* and *CALR* mutations was performed for all the patients included in this study. All other data were collected from electronic medical records in our institution.

Detection Of *JAK2* Mutations

The bone marrow genomic DNA was obtained and used for PCR amplification with the following primers: *JAK2*-Exon14-F (GGACCAAAGCACATTGTATCCTC) and *JAK2*-Exon14-R (GGGCATTGTAACTTCTACTT). The resulting 400-bp *JAK2* PCR product was purified using a PCR Purification Kit (Qiagen, Valencia, CA). Quantitative allele-specific suppressive PCR was performed using the purified PCR product on a sequence detection system 7000 platform (Applied Biosystems, Foster City, CA) as previously

described [19]. A threshold of 1% was used to determine the presence of a *JAK2* (V617F) mutation. For one *JAK2* positive patient this test was repeated on bone marrow DNA collected 9 and 12 years prior to the diagnosis of MPN, due to concomitant CLL diagnosis.

Detection Of *CALR* Mutations

To screen for insertion and deletion mutations in *CALR* exon 9, we used PCR primers for exon 9 and labeled the forward primer with fluorescein probe, as previously described[13]. After PCR, the products were sized by Gene Scan. A single band of 260 bp was expected in the wild-type gene. Detection of bands of different size was considered predictive of *CALR* mutation (insertion or deletion). All samples identified as having a mutation were confirmed by Sanger sequencing.

Detection Of *MPL* Mutations

To detect mutations in exon 10 of *MPL*, we amplified genomic DNA using the following primer set: *MPL*13474-F (GTGACCGCTCTGCATCTAGTG) and *MPL*13726-R (GTGGGCGTGTTAGAGTGT). The resulting 250-bp PCR product was purified using a PCR purification kit (Qiagen) and was subjected to Sanger sequencing using the reverse primer on a 3300 genetic analyzer (Applied Biosystems). The DNA sequencing fragments were analyzed using DNASTAR Lasergene 11. Details on cytogenetic, fluorescence in situ hybridization (FISH), somatic mutation of the immunoglobulin heavy chain (IGHV) variable gene are included into the supplemental material section.

Statistical Analysis

Categorical variables were compared using the Fisher exact test characteristics. Survival curves were generated by the Kaplan-Meier method. Overall survival (OS) was calculated from date of diagnosis of the first hematological disease, CLL or MPN to death or the date of last follow-up. Impact on survival of clinical variables was evaluated by comparing the entire OS curves by means of the log-rank test. All the probability (P) values were 2-sided and considered significant if ≤ 0.05 . All statistical analyses were carried out using IBM SPSS Statistics 22 for Windows.

RESULTS

Patient Characteristics

Thirteen patients with concomitant MPN and CLL were seen in MD Anderson's leukemia clinic between the years 1998 and 2014. The median age at first diagnosis was 60 years (range 42-75). Nine of the 13 (69%) were male. All patients were white. Three patients (23%) had a family history of hematological malignancies among their first-degree relatives. The median time of follow-up was 9.7 years (range 0.7-19.1). In 7 patients the diagnosis of CLL preceded that of MPN, in 5 patients the diagnosis of MPN preceded the diagnosis of CLL, and in one patient CLL and PMF were synchronously diagnosed.

The median age at the time of CLL diagnosis was 67 years (range 43-83). Six patients (46%) had fatigue but no other B-symptoms, and all other patients were asymptomatic. Five patients (38%) had Rai stage 0 disease, 4 (31%) had stage I, 2 had stage II, one had stage III,

and another patient had Rai stage IV disease. The median OS from diagnosis was 9.3 years. The patients' clinical characteristics and cytogenetic, FISH, and *IgHV* gene somatic mutation status are summarized in Table I. During follow-up, a total of 7 patients experienced progression to Rai stage III/IV and all, except one patient who had severe heart failure, were treated. The median time-to-first treatment was 15 months (range 3-58) and the overall response rate was 83%. The median OS of these patients was 7.1 years, while that of the untreated patients was 9.3 years. Within this treated subgroup, 5 patients received treatment before developing PMF and one after diagnosis of PV (Table I).

The median age at the time of MPN diagnosis was 63.3 years (range 56.2-76.9). Twelve (92.3%) of the thirteen patients had constitutional symptoms. Eight (61.5%) of the 13 patients had PMF, 2 had PV, and 3 had ET. All patients with PV or ET experienced progression to post-PV or post-ET myelofibrosis (MF) after a median of 9.7 years (range 0.8-19) after diagnosis. The median OS from the onset of PMF was 2.8 years, from the onset of PV 11 years, and from the onset of ET 9.6 years. At diagnosis of PMF, one patient was low-risk according to the International Prognostic Score System (IPSS) [20], 4 patients were intermediate-1 risk, and 3 patients were intermediate-2 risk; none of the patients had high-risk disease. Five (38%) of the 13 patients were treated with hydroxyurea, one was treated with ruxolitinib, 2 with lenalidomide, and 5 (38%) did not receive any specific treatment for MPN (Table I).

Five (38%) MPN patients had a *JAK2* (V617F) mutation, 3 had a *CALR* mutation, and no patient had an *MPL* W515L/K mutation. All mutations were mutually exclusive. Five (38%) patients did not carry any of these mutations and were classified as "triple negative." Of the 8 PMF patients, 3 (37%) had a *JAK2* mutation, one had a *CALR* mutation, and 4 (50%) were triple negative. Both PV patients carried a *JAK2* mutation. Of the ET patients, 2 carried a *CALR* mutation, whereas the other was triple negative. Sanger sequencing identified a type-1 mutation consisting of a 52-bp deletion in exon 9 of the *CALR* gene in all patients with a *CALR* mutation.

MPN Type, Disease Onset And Survival

In our series PMF, but not PV or ET, occurred in 7 patients (2 of whom were previously untreated) who were followed up or treated for CLL and in one treatment-naïve patient in whom PMF and CLL were concomitantly diagnosed; conversely, PV and ET, but not PMF, preceded CLL ($P=0.001$). All PV and ET patients experienced progression to MF, and CLL was diagnosed on BM aspirates during progression to post-PV or post-ET MF. The median OS from first hematologic diagnosis of patients with CLL preceding MPN (PMF) was 9.3 years, whereas that of patients with MPN preceding CLL was 11 years. The median age at first hematologic diagnosis was 63.8 years in the first group and 59.3 years in the second one.

Chromosomal Abnormalities And The Development Of MPN In Patients With CLL

We explored the correlation between genetic lesions and previous potentially leukemogenic therapy by comparing karyotype and disease-specific mutation at the time of CLL and MPN diagnoses. Mutations in *JAK2* or *CALR* were found in both untreated and previously treated

patients. Patients treated with hydroxyurea did not develop additional chromosomal abnormalities when reassessed for subsequent CLL diagnosis. Conversely, 2 of the 5 CLL patients who required treatment developed additional chromosomal abnormalities that are usually not found in patients with CLL, and one patient had a del 5q (usually not detected in CLL) when diagnosed with CLL, suggesting the pre-existence of MPN (Table I).

JAK2 Mutation Status In A Patient With CLL Preceding PMF

DNA analysis of patient #6 provides some insight into the pathogenesis of MPN in CLL. CLL Rai stage I was diagnosed in this patient in 1998. After 2 years, his disease progressed to Rai stage IV and he was treated with FCR for 6 courses. The patient attained complete remission and was doing well. Twelve years later, he developed anemia, and BM biopsy showed relapsed CLL and a *JAK2*-mutated PMF. In DNA retrieved from archived BM specimens from the time of diagnosis, 9 and 12 years prior to the diagnosis of PMF, a *JAK2* mutation was not detected at the time CLL was diagnosed but was readily detected (allelic burden=1.3%) 2 years after the initiation of chemotherapy. Nine years later, the patient developed symptomatic PMF with a higher *JAK2* mutation allelic burden (23.5%). Treatment with ruxolitinib was initiated, with improvement of PMF-related symptoms. Treatment with ruxolitinib was ongoing at the last follow-up and, after 15 months, no lymphocytosis or lymphadenopathy was detectable despite CLL relapse.

DISCUSSION

Our study provides clinical, biological, and prognostic characteristics of 13 patients with concomitant CLL and MPN seen over a 17-year period. Retrospective study design and small sample size are the major limitation of this study. The study design does not allow further pathogenic characterization of statistical associations described above. Due to the retrospective study design, some data are missed; this mainly limits IGHV mutational status analysis because this evaluation has been introduced into the clinical practice since 2003. In spite of these limitations, some features found in our patients suggest that CLL and MPN pathobiological processes might be intertwined and their co-occurrence might be not stochastic. Indeed, the prevalence of CLL in the United States is 40 per 100,000 persons and that of MPN is 115 per 100,000 citizens [1-3], and the expected prevalence of the occurrence of both diseases in a single individual is 4.6×10^{-8} . We could not find any epidemiological data on CLL coinciding with MPN in the general population but, considered the very low joint probability, prevalence of concomitant CLL/MPN in our case-series is higher than expected. When occurring with MPN, CLL seems to have some peculiar characteristics. In 54% of the patients in our study, CLL progressed to Rai stage III/IV disease, higher than the incidence usually observed in patients with CLL [21]. The prevalence of 13q cytogenetic abnormality and normal karyotype in our series were 70% and 0%, respectively, while they were 42% and 27% in a previously published study of CLL patients [21]. Del 11q, known to be associated with increased reticulin BM fibrosis in patients with CLL [22], was detected only in PV patients. These clinico-biological features and the higher-than-expected joint frequency support the hypothesis that CLL/MPN co-occurrence might hide a common pathobiological pathway. Given the relatively small sample no conclusion could be drawn. However, these findings should be further explored. A prospective study would overcome

the limitations of our (and previous) study and would allow to identify the molecular fingerprint of malignant myeloid and lymphoid cell population.

Although PMF preceding CLL and CLL preceding PV or ET were previously reported [8], in our series we found an association between the MPN type (PMF vs PV/ET) and timing of co-occurrence diagnosis (CLL prior to MPN vs MPN prior to CLL). The median OS from the time of MPN diagnosis was shorter than previously reported in each MPN category (the OS in our PMF patients was 2.8 vs. reported 4.5 years, in our PV patients 11 vs. reported 14 years, and in our ET patients 9.6 vs reported 14.7 years) [10, 23, 24], although the incidence and type of chromosomal abnormalities were not different from those previously reported [25]. The OS of our CLL patients, however, is similar to historical data [26].

Two hematological neoplasms such as CLL and MPN might coincide incidentally or as a result of a common etiology, such as a hereditary neoplastic syndrome or risk factors active at the early stage in the pathogenesis of both diseases [27]. Whereas leukemogenic mutations might be acquired stochastically [28], environmental and genetic predisposition factors could enhance mutagenesis in self-renewing progenitors, leading to genetic instability and the development of concomitant diseases [29]. Although the current dogma is that CLL and MPN arise from diverse committed progenitor cells [12], an alternative possibility is that an early event in a common stem/progenitor cell might contribute to the occurrence of CLL and MPN by progressive accumulation of genetic and epigenetic changes leading to genetic instability. Recent studies showed increased expression of early lymphoid transcription factors, such as IKAROS, and somatic mutations in NOTCH1 and SF3B1 in early hematopoietic cells in CLL patients [30, 31]. Whole-exome and whole-genome sequencing in MPN and CLL discovered recurrent mutations in both diseases [13-16]. Some of the somatic mutations might predispose hemopoietic stem/progenitor cells to acquire additional aberrations that are detected across different hematologic malignancies [32]. However, since no common genomic mutations have been identified between CLL and MPN, further studies should be looking for common epigenetic aberrations which might explain the constitutive JAK/STAT pathway activation both in CLL and triple negative MPN.

We detected an activating *JAK2* mutation in a patient with CLL 9 years before he developed PMF. Over the years the levels of the mutated *JAK2* allele burden significantly increased. Previous studies on genetic characterization of MPN burst-forming unit-erythroid colonies [19, 33] suggested that the *JAK2* mutation is not a disease-initiating event. Our data are consistent with the hypothesis that a complex sequence of genetic and epigenetic aberrations must be acquired together with JAK/STAT pathway aberrations before a clinical MPN occurs. However, the polymorphic MPN clinical phenotype and the underlying genetic heterogeneity make the 2-hit hypothesis for cancer development [34] too simplistic to explain the MPN development. Alternatively, the *JAK2*-positive clone could have harbored the mutations needed to sustain independent growth and survival, but could not escape immune surveillance.

Notably, a paradoxical deregulation affects the immune system in both CLL [35] and MPN patients [36], causing autoimmunity abnormalities as well as impaired response to infectious stimuli. There are no published data exploring whether these immunologic aberrations

interact with or affect the development of concomitant MPN and CLL. Nonetheless, despite the low incidence of co-occurrence, several observations suggest that a common pathobiological mechanism for CLL and MPN does exist. A relative increase in CD5+ B-lymphocytes was found in 10% of patients with PMF without any clinical evidence of CLL [37]. One third of CLL patients have increased BM reticulin fibrosis [22]. In addition, similar signaling pathways are activated in both neoplasms; STAT3 is constitutively activated in CLL, stimulation of the B-cell receptor activates *JAK2* in CLL cells [38], and *JAK2*, known to activate the STAT pathway, is constitutively activated in MPN [39, 40].

Our retrospective, small sample size analysis suggests that a link between these two clinical entities exists. Further studies on concomitantly occurring CLL and MPN might reveal early pathogenic event(s) promoting and sustaining both lymphoid and myeloid neoplasms. Therefore prospective longitudinal studies of patients with these concomitantly occurring neoplasms are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT AND DECLARATION OF INTERESTS

This study was supported by a grant from the CLL Global Research Foundation.

The University of Texas MD Anderson Cancer Center is supported in part by the National Institutes of Health through a Cancer Center Support Grant (P30 CA16672).

REFERENCES

1. Call TG, Phylly RL, Noel P, et al. Incidence of chronic lymphocytic leukemia in Olmsted County, Minnesota, 1935 through 1989, with emphasis on changes in initial stage at diagnosis. *Mayo Clinic proceedings*. 1994; 69:323–328. [PubMed: 8170175]
2. Mehta J, Wang H, Iqbal SU, et al. Epidemiology of myeloproliferative neoplasms in the United States. *Leukemia & lymphoma*. 2014; 55:595–600. [PubMed: 23768070]
3. Siegel R, Ma J, Zou Z, et al. Cancer statistics, 2014. *CA: a cancer journal for clinicians*. 2014; 64:9–29. [PubMed: 24399786]
4. Kodali S, Chen C, Rathnasabapathy C, et al. *JAK2* mutation in a patient with CLL with coexistent myeloproliferative neoplasm (MPN). *Leukemia research*. 2009; 33:e236–239. [PubMed: 19625083]
5. Salama ME, Swierczek SI, Tashi T, et al. Calreticulin mutated prefibrotic-stage myelofibrosis and PMF represent an independent clone from coexisting CLL. *Blood*. 2014; 124:1691–1692. [PubMed: 25190754]
6. Swierczek S, Nausova J, Jelinek J, et al. Concomitant *JAK2* V617F-positive polycythemia vera and B-cell chronic lymphocytic leukemia in three patients originating from two separate hematopoietic stem cells. *American journal of hematology*. 2013; 88:157–158. [PubMed: 23280542]
7. Kim H, Jang W, Shin S, et al. Two cases of concurrent development of essential thrombocythemia with chronic lymphocytic leukemia, one related to clonal B-cell lymphocytosis, tested by array comparative genomic hybridization. *International journal of hematology*. 2014
8. Laurenti L, Tarnani M, Nichele I, et al. The coexistence of chronic lymphocytic leukemia and myeloproliferative neoplasms: a retrospective multicentric GIMEMA experience. *American journal of hematology*. 2011; 86:1007–1012. [PubMed: 21953617]
9. Fianchi L, Pagano L, Piciocchi A, et al. Characteristics and outcome of therapy-related myeloid neoplasms: Report from the Italian network on secondary leukemias. *American journal of hematology*. 2015

10. Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*. 2010; 115:1703–1708. [PubMed: 20008785]
11. Rumi E, Passamonti F, Elena C, et al. Increased risk of lymphoid neoplasm in patients with myeloproliferative neoplasm: a study of 1,915 patients. *Haematologica*. 2011; 96:454–458. [PubMed: 21109692]
12. Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *The American journal of pathology*. 2006; 169:338–346. [PubMed: 16877336]
13. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *The New England journal of medicine*. 2013; 369:2379–2390. [PubMed: 24325356]
14. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *The New England journal of medicine*. 2013; 369:2391–2405. [PubMed: 24325359]
15. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011; 475:101–105. [PubMed: 21642962]
16. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nature genetics*. 2012; 44:47–52. [PubMed: 22158541]
17. Swerdlow, SH.; Harris, NL., et al. World Health Organization Classification of Tumours of Hematopoietic and Lymphoid Tissues. 4th. IARC Press; 2008. CE
18. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood*. 2008; 111:5446–5456. [PubMed: 18216293]
19. Nussenzveig RH, Swierczek SI, Jelinek J, et al. Polycythemia vera is not initiated by JAK2V617F mutation. *Experimental hematology*. 2007; 35:32–38. [PubMed: 17198871]
20. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009; 113:2895–2901. [PubMed: 18988864]
21. Wierda WG, O'Brien S, Wang X, et al. Multivariable model for time to first treatment in patients with chronic lymphocytic leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; 29:4088–4095. [PubMed: 21969505]
22. Tadmor T, Shvidel L, Aviv A, et al. Significance of bone marrow reticulin fibrosis in chronic lymphocytic leukemia at diagnosis: a study of 176 patients with prognostic implications. *Cancer*. 2013; 119:1853–1859. [PubMed: 23423815]
23. Passamonti F, Thiele J, Girodon F, et al. A prognostic model to predict survival in 867 World Health Organization-defined essential thrombocythemia at diagnosis: a study by the International Working Group on Myelofibrosis Research and Treatment. *Blood*. 2012; 120:1197–1201. [PubMed: 22740446]
24. Tefferi A, Rumi E, Finazzi G, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. *Leukemia*. 2013; 27:1874–1881. [PubMed: 23739289]
25. Tefferi A. Myelofibrosis with myeloid metaplasia. *The New England journal of medicine*. 2000; 342:1255–1265. [PubMed: 10781623]
26. Pflug N, Bahlo J, Shanafelt TD, et al. Development of a comprehensive prognostic index for patients with chronic lymphocytic leukemia. *Blood*. 2014; 124:49–62. [PubMed: 24797299]
27. Adams AM, Hudson RR. Maximum-likelihood estimation of demographic parameters using the frequency spectrum of unlinked single-nucleotide polymorphisms. *Genetics*. 2004; 168:1699–1712. [PubMed: 15579718]
28. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science (New York, NY)*. 2015; 347:78–81.

29. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
30. Damm F, Mylonas E, Cosson A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. *Cancer Discovery*. 2014; 4:1088–1101. [PubMed: 24920063]
31. Kikushige Y, Ishikawa F, Miyamoto T, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. *Cancer Cell*. 2011; 20:246–259. [PubMed: 21840488]
32. Ylipaa A, Yli-Harja O, Zhang W, et al. Characterization of aberrant pathways across human cancers. *BMC systems biology*. 2013; 7(Suppl 1):S1. [PubMed: 24267866]
33. Ortmann CA, Kent DG, Nangalia J, et al. Effect of mutation order on myeloproliferative neoplasms. *The New England journal of medicine*. 2015; 372:601–612. [PubMed: 25671252]
34. Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America*. 1971; 68:820–823. [PubMed: 5279523]
35. Rozovski U, Keating MJ, Estrov Z. Targeting inflammatory pathways in chronic lymphocytic leukemia. *Critical reviews in oncology/hematology*. 2013; 88:655–666. [PubMed: 23941728]
36. Barosi G. An immune dysregulation in MPN. *Current hematologic malignancy reports*. 2014; 9:331–339. [PubMed: 25139710]
37. Cervantes F, Hernandez-Boluda JC, Villamor N, et al. Assessment of peripheral blood lymphocyte subsets in idiopathic myelofibrosis. *European journal of haematology*. 2000; 65:104–108. [PubMed: 10966169]
38. Rozovski U, Wu JY, Harris DM, et al. Stimulation of the B-cell receptor activates the JAK2/STAT3 signaling pathway in chronic lymphocytic leukemia cells. *Blood*. 2014; 123:3797–3802. [PubMed: 24778152]
39. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood*. 2010; 116:988–992. [PubMed: 20404132]
40. Teofili L, Martini M, Cenci T, et al. Different STAT-3 and STAT-5 phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F JAK-2 mutation. *Blood*. 2007; 110:354–359. [PubMed: 17376889]

Table I

Clinical and biological characteristics at CLL and MPN diagnosis (n=13)

#	Age (years)	MPN type	Interval between CLL and MPN Dx (years)	Karyotype at CLL Dx	FISH	IGHV mutation status	Chemo-therapy*	MPN associated mutation	Karyotype at MPN Dx	Survival (years)	Status
Patients with CLL preceding MPN (n=7)											
1	60	PMF	2.7	normal	del13q14	Unmut	CTX	triple negative	normal	7.1	Dead
2	70	PMF	6.5	normal	del13q14	Mut	-	triple negative	normal	9.3	Dead
3	67	PMF	5.6	normal	del13q14	Mut	B	triple negative	del 7	8.5	Alive
4	42	PMF	13.6	normal	not available	Unmut	R-CHOP; FR	triple negative	normal	13.7	Alive
5	50	PMF	13.1	t (3;12)	del13q14	Mut	-	<i>CALR</i>	t (3;12)	15.7	Alive
6	48	PMF	13.3	del 20q11	not available	not available	FCR	<i>JAK2</i>	del 8q21 del 20q11	15.1	Alive
7	75	PMF	1	del 5q del 13q	del13q14	not available	BR	<i>JAK2</i>	del 5q del 13q	1.2	Dead
Patients with synchronous CLL and MPN (n=1)											
8	71	PMF	0	not available	+12	Mut	-	<i>JAK2</i>	not available	0.7	Dead
Patients with MPN preceding CLL (n=5)											
9	59	ET	7	normal	not available	Mut	HU	triple negative	normal	9.6	Dead
10	58	ET	0.8	normal	+ 12, del13q14	Unmut	HU	<i>CALR</i>	normal	8.1	Alive
11	59	ET	19	not available	not available	not available	lenalidomide	<i>CALR</i>	normal	19.1	Alive
12	70	PV	13	add 18q23	del 11q, del13q14	not available	HU	<i>JAK2</i>	add 18q23	18.5	Alive
13	59	PV	9.7	+12 del 20q11	del 11q, +12	not available	HU	<i>JAK2</i>	+12 del 20q11	11.1	Dead

MPN, myeloproliferative neoplasms; CLL, chronic lymphocytic leukemia; Dx, diagnosis; FISH, fluorescence in situ hybridization; IgHV, immunoglobulin heavy chain variable region; PMF, primary myelofibrosis; Unmut, unmutated; CTX, cyclophosphamide; Mut, mutated; B, bendamustine; R-CHOP, rituximab-cyclophosphamide-adriamycin-vincristine-prednisone; FR, fludarabine-rituximab; FCR, fludarabine-cyclophosphamide-rituximab; BR, bendamustine-rituximab; ET, essential thrombocythemia; PV, polycythemia vera; HU, hydroxyurea.

* Only chemotherapy regimens administered between MPN and CLL onset are shown in this table.