How I Treat Atypical Chronic Myeloid Leukemia

Jason Gotlib

1Division of Hematology, Stanford University School of Medicine / Stanford Cancer Institute, Stanford, CA

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Corresponding Author
Jason Gotlib, MD, MS
Professor of Medicine (Hematology)
Stanford Cancer Institute
875 Blake Wilbur Drive, Room 2324
Stanford, CA 94305-5821

TEL:  650-736-1253
FAX:  650-724-5203
Email:  jason.gotlib@stanford.edu

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Abstract

Atypical chronic myeloid leukemia, *BCR-ABL1*-negative (aCML) is a rare myelodysplastic syndrome/myeloproliferative neoplasm for which no current standard of care exists. The challenges of atypical CML relate to its heterogeneous clinical and genetic features, high rate of transformation to acute myeloid leukemia, and historically poor survival. Therefore, allogeneic hematopoietic stem cell transplantation should always be an initial consideration for eligible patients with a suitable donor. Non-transplant approaches for treating aCML have otherwise largely relied on adopting treatment strategies used for MDS and MPN. However, such therapies, including hypomethylating agents, are based on a paucity of data. With an eye toward making a more meaningful impact on response rates and modification of the natural history of the disease, progress will rely on enrollment of patients into clinical trials and molecular profiling of individuals so that opportunities for targeted therapy can be exploited.
Introduction

The current diagnostic criteria that comprise the World Health Organization (WHO) entity ‘atypical chronic myeloid leukemia, BCR-ABL1 negative’ (Table 1) represents a decades-long evolution of classifying diseases which exhibited morphologic similarity to chronic myeloid leukemia (CML), but lacked both the Philadelphia (Ph) chromosome by standard cytogenetics and BCR-ABL1 rearrangement by polymerase chain reaction (PCR).1,2 The differential diagnosis of these BCR-ABL1-negative hematologic neoplasms not only includes atypical CML, but also chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia (CNL), and MDS/MPN, unclassifiable (MDS/MPN, U).1-6 The knowledge gleaned from next generation sequencing has complemented morphologic and laboratory WHO criteria for myeloid neoplasms, and can often provide greater specificity in distinguishing atypical CML from alternative MDS/MPN or MPNs.1,3-7 Invariably, how I pursue the diagnosis and treatment atypical CML requires attention to the results of standard cytogenetic analysis and myeloid mutation testing since druggable targets may be unmasked.

Case 1

LJ is a 62-year-old woman with a past medical history of hyperlipidemia and a left total hip replacement. In the last 3 months, during two episodes of diverticulitis with associated gastrointestinal bleeding requiring red blood cell transfusion support, a new leukocytosis of 15-20 x 10^9/L was identified. The platelet count was normal and a manual differential revealed 46% neutrophils, 12% band forms, 12% metamyelocytes, 6% monocytes, 6% myelocytes, 2% promyelocytes, 2% eosinophils, and 14%
lymphocytes. The increased white blood cell count and left-shifted white blood cell
differential was felt to be reactive due to her acute medical condition. The patient was
seen in consultation in hematology clinic two months after hospital discharge because of
persistent blood count abnormalities despite resolution of her gastrointestinal issues. No
hepatosplenomegaly was noted on examination. A complete blood count (CBC) showed
persistent elevation of the WBC count to $24.2 \times 10^9$/L, hemoglobin of 11.2 g/dL, platelet
count $160 \times 10^9$/L with a similar spectrum of myeloid immaturity. A bone marrow
aspirate and biopsy revealed hypercellularity for age, left-shifted myeloid hyperplasia
without increased blasts, and hypogranular granulocytes with abnormal nuclear
segmentation. Dyserythropoiesis and dysplastic megakaryocytes, including hypolobated
forms, were noted. Cytogenetics were normal and PCR for $BCR-ABL1$ was negative. A
diagnosis of atypical CML was made. The patient was referred for evaluation of
management options.

**Case 2**

CK is a 76 year-old man with a history of coronary artery disease and hepatitis C that had
been treated with pegylated-interferon-alpha-2a (PEG-IFN-alpha-2a) until 1.5 years ago
when it was discontinued because of progressive depressive symptoms and cytopenias.
After 4 months off therapy, the patient reported increasing fatigue and new night sweats.
A spleen tip was palpated on examination. The WBC count increased from $2.5 \times 10^9$/L to
$26.2 \times 10^9$/L after PEG-IFN-alpha-2a was stopped; the hemoglobin was 10.6 g/dL, and
the platelet count was $133 \times 10^9$/L. Although an automatic differential showed 89%
neutrophils, a manual differential revealed 17% neutrophils, 27% band forms, 6%
metamyelocytes, 8% monocytes, 15% myelocytes, 14% promyelocytes, 1% blasts, and 11% lymphocytes. Review of the peripheral blood and bone marrow aspirate revealed an increased number of left-shifted leukocytes with hypolobation and pseudo Pelger-Huët morphologies. A bone marrow aspirate was hypercellular without increased blasts; there was subtle dyserythropoiesis, and dysmegakaryopoiesis primarily consisting of hypolobated megakaryocytes with separate nuclear lobes. The bone marrow biopsy was hypercellular (95%) with a M:E ratio of 5:1. Cytogenetics showed trisomy 8, and no Ph chromosome. The patient was referred for a second opinion. Pathology was confirmed, and next generation sequencing revealed \( \text{CSF3R} \) T618I (43% mutant allele frequency) and \( \text{U2AF1} \) Q157T (48% mutant allele frequency) mutations. Treatment options were reviewed.

Some cases of atypical CML have been given the historical moniker ‘CML-like syndrome’, because both diseases exhibit bone marrows with hyperplastic myeloid hyperplasia and peripheral blood leukocytosis characterized by a spectrum of myeloid immaturity. However, on morphologic grounds, this is where the similarity ends. Unlike \( \text{BCR-ABL1} \)-positive CML, atypical CML is characterized by prominent dysplastic granulopoiesis, (e.g. the acquired Pelger-Huët anomaly; nuclear abnormalities including hypersegmentation, nuclear projections, and abnormally clumped nuclear chromatin; and abnormalities of cytoplasmic granules, such as hypogranularity), and in some cases multilineage dysplasia may be observed.\(^1\,\,^2\) The finding of \( \geq 10\% \) immature myeloid cells (promyelocytes, myelocytes, and metamyelocytes) in the peripheral blood and/or dysplasia are useful criteria in distinguishing aCML from CNL, which lacks these
features. Additional features of aCML include absent or minimally present basophilia (<2% of leukocytes) and monocytosis (<10% of leukocytes) which are additional morphologic findings that help distinguish atypical CML from BCR-ABL1-positive CML and CMML, respectively.¹,²

**Natural History and Prognostic Factors**

In an Italian cohort of 55 WHO-defined aCML cases,⁸ the overall median survival was 25 months compared to survivals of 14-30 months derived from three smaller studies.⁹⁻¹¹ A recent U.S. multicenter study applied WHO 2008 criteria to compare aCML (n=65) and MDS/MPN, U (n=69) cases, and found that the former exhibited a more aggressive clinical course, with respective median overall survivals of 12.4 and 21.8 months.⁶ In the U.S. and Italian studies,⁷,⁸ transformation to acute myeloid leukemia (AML) occurred in 37% and 40% of the patients, with a median time to transformation of 11.2 and 18 months, respectively. Increased WBC count (e.g. cutoffs of > 40 or 50 x 10⁹/L), increased percentage of peripheral blood immature precursors, female sex, and older age have been shown to be adverse prognostic factors for overall survival or leukemia-free survival in multivariate analyses.⁷,⁸

**Molecular and Cytogenetic Features**

In cases where subtle dysplasia or borderline levels of myeloid immaturity or monocytosis are present, morphologic distinction between aCML, BCR-ABL1-positive CML, CMML, CNL, or MDS/MPN-U can be challenging. In addition to histopathologic analysis of the peripheral blood and bone marrow, modern evaluation of
atypical CML should include next generation sequencing vis-à-vis myeloid mutation panel testing in addition to standard karyotyping.\textsuperscript{1-7,12} Diagnosis of aCML first requires testing for the Philadelphia chromosome and/or the \textit{BCR-ABL1} fusion gene to exclude CML.\textsuperscript{1,2} Standard karyotyping, fluorescent \textit{in-situ} hybridization, and myeloid mutation testing not only complement morphologic analyses, but may also identify opportunities for targeted therapy (\textbf{Figure 1}).

In contrast to \textit{BCR-ABL1}, which operationally defines CML, no single genetic lesion characterizes aCML. The mutations identified in aCML are commonly found in other myeloid neoplasms.\textsuperscript{3-7,12-15} The variability that exists in the reported frequency of specific mutations in aCML may partly reflect the stringency to which the WHO definition of aCML was applied in different publications.\textsuperscript{3} However, some basic observations can be made about the molecular landscape of aCML: higher frequency mutations (e.g. >20\%) include \textit{SETBP1}, \textit{ASXL1}, \textit{N/K-RAS}, \textit{SRSF2}, and \textit{TET2}, and lower frequency mutations (<10\%) include \textit{CBL}, \textit{CSF3R}, \textit{JAK2}, and \textit{ETNK1}.\textsuperscript{3-7,12-15}

Recurrent \textit{SETBP1} mutations have been identified in ~25-33\% of aCML patients and represent one of the mostly frequently mutated genes in this disease.\textsuperscript{13-15} Set binding protein (SETBP1) interacts with SET, a negative regulator of the tumor suppressor protein phosphatase 2A (PP2A).\textsuperscript{16} SETBP1 protects SET from protease cleavage, resulting in an increased amount of SET available to repress PP2A activity.\textsuperscript{17}

Most \textit{SETBP1} mutations are located within a 14-amino acid stretch (codons 858-871) which is also mutated in Schinzel-Giedion syndrome, a rare genetic disease characterized
by congenital malformations, mental retardation, and frequent epithelial tumors.\textsuperscript{18} Normally, phosphorylation of this region leads to binding by E3 ubiquitin ligase subunit b-TrCP1, resulting in ubiquitination and subsequent degradation of \textit{SETBP1}.\textsuperscript{13} \textit{SETBP1} mutants disrupt this consensus b-TrCP motif, leading to increased \textit{SETBP1} and SET expression, which decreases PP2A activity and increases cellular proliferation.\textsuperscript{13}

\textit{SETBP1} mutations are associated with a higher leukocyte count, lower hemoglobin and platelet counts, and worse overall survival.\textsuperscript{13,14} In one study, \textit{SETBP1} mutations were associated with the presence of -7 and isochromosome i(17)(q10) cytogenetic abnormalities as well as \textit{ASXL1} and \textit{CBL} mutations, but were mutually exclusive of mutations in the \textit{TET2} and \textit{JAK2} genes.\textsuperscript{14} However, \textit{SETBP1} is a ubiquitous molecular abnormality among myeloid neoplasms, including CNL and CMML, and may be found in tandem with other mutations, such as with \textit{CSF3R} in cases of CNL.\textsuperscript{3,6,14,15,19}

Although originally reported at a higher frequency in aCML,\textsuperscript{20} subsequent reports indicate that the activating \textit{CSF3R} T618I mutation is present in $< 10\%$ of cases.\textsuperscript{1,3,7,12,15} Identification of \textit{CSF3R} T618I in the context of neutrophilic leukocytosis strongly favors a diagnosis of CNL where it is present in approximately 80\% of patients.\textsuperscript{1,3,20,21} Although T618I is the most common activating mutation in \textit{CSF3R}, an alternative proximal membrane mutation, T640N, has been described in a case of MDS that exhibited transformation to a secondary aCML-like picture.\textsuperscript{22} Rarely, nonsense and frameshift mutations that result in truncation of the cytoplasmic tail of \textit{CSF3R} have been found in cases of aCML, and are similar to those identified in patients with severe
congenital neutropenia who have been administered G-CSF therapy. Evolution of a case of MPN-U to a phenotype of aCML was associated with new subclones of both CSF3R proximal membrane (T618I, 35% mutant allele frequency) and truncation (Q739*, 30% mutant allele frequency) mutants.

JAK2 V617F is an uncommon mutation (3-7%) in aCML and its identification (as well as similar Ph’ MPN-associated mutations in CALR and MPL), tends to favor an alternative diagnosis such as PV, ET, or MF in the appropriate clinicopathologic context. More recently, mutations in the ethanolamine kinase 1 (ETNK1) gene were found in 9% of aCML cases, and were also enriched in patients with CMML and systemic mastocytosis with associated eosinophilia. KRAS/NRAS mutations were identified in 7/20 (35%) aCML patients in the aforementioned U.S. multicenter analysis. Although SETBP1 and ETNK1 mutations are not yet druggable targets, mutated CSF3R, JAK2, and RAS are important to identify since clinical trial or off-label opportunities for targeted therapy against these lesions may be available (see below; and Figures 1 and 2).

Non-specific karyotypic abnormalities have been reported in a moderate proportion of aCML patients. These include single or double abnormalities, or complex cytogenetics, including trisomy 8, del(20q), -7/7q- or isochromosome 17q [i17(q)]. Notably, the literature includes patients with rearrangement of PDGFRα, PDGFRβ, FGFR1, or PCM1-JAK2 who have been given the diagnosis of aCML. While in some of these cases the term ‘atypical CML’ may have been loosely applied to indicate a CML-like disease in the absence of BCR-ABL1, other cases may truly fulfill morphologic criteria.
for aCML. However, according to the WHO classification, the presence of any of these genetic rearrangements re-assigns such cases to the major category of ‘Myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of PDGFA, PDGFRB, FGFR1 or PCM1-JAK2’. 1,2 Although not formally included in this category, cases with rearranged FLT3 may rarely morphologically mimic aCML. 3,1 Recognizing the relevant breakpoints for reciprocal translocations that infer involvement of PDGFA (4q12; excluding the most common FIP1L1-PDGFA rearrangement which is not visible by standard karyotyping), PDGFRB (5q31~33), FGFR1 (8p11), and JAK2 (9p24) is also critical for recognizing instances where use of tyrosine kinase inhibitors such as imatinib should be considered (e.g. rearranged PDGFA/B with confirmation by FISH or sequencing), or where poor disease prognosis disease mandates high-intensity approaches such as induction chemotherapy and/or allogeneic hematopoietic stem cell transplantation (HSCT) (e.g. for patients with rearranged FGFR1).

**Treatment**

No standard of care exists for the treatment of atypical CML. In addition, no consensus recommendations or risk-based treatment algorithms exist to help guide a watch-and-wait approach versus initiation of therapy. However, progressive leukocytosis, anemia and/or thrombocytopenia, or emergence of symptomatic splenomegaly or disease-related constitutional symptoms should prompt treatment.

Given its unfavorable prognosis, my treatment algorithm (Figure 2) is first to consider HSCT for eligible patients with a suitable donor without initially relying on the results of
myeloid mutation testing. Although the best timing of transplantation, e.g. earlier in the
course of disease, or at the time of disease progression, remains an unresolved question,
the otherwise poor outcomes of aCML patients should encourage evaluation of the
feasibility of this treatment modality after the diagnosis is made. If a donor is not
immediately available and/or disease cytoreduction is recommended, I consider the
results of myeloid mutation testing to evaluate clinical trial options (preferred) or off-
label opportunities with targeted therapy. Current examples include ruxolitinib for
CSF3R or JAK2-mutated patients, or MEK inhibition in RAS-mutated patients (see
below, future prospects). Regardless of the results of mutation testing, hypomethylating
therapy may be considered in such individuals since the prognostic relevance of these
mutations to treatment response is unknown. I similarly use the results of myeloid
mutation panel testing for patients who are not transplant candidates to evaluate
opportunities for trials of targeted therapy. If no such option exists, my approach is to
consider hypomethylating therapy, or clinical trials of novel therapies not based on an
actionable mutation. Additionally, I co-opt treatment strategies used for either MDS or
MPN and apply them on a case-by-case basis to address a patient’s major clinical issues
(e.g. leukocytosis, anemia, constitutional symptoms, splenomegaly, and potential for
progression to AML). These second-line or adjunctive options may include pegylated-
interferon-α, hydroxyurea, and/or erythropoiesis stimulating agents.

**Hematopoietic stem cell transplantation (HSCT)**

A limited number of HSCT procedures for aCML have been published. Most are
included in series of patients with heterogeneous MDS/MPN where long-term disease
free-survival of 40-50% has been recorded. Koldehoff and colleagues’ retrospective analysis of 9 individuals with aCML represents the largest transplantation series focused solely on this disease. In this series, allogeneic donor types consisted of an HLA-identical sibling (N=4), or unrelated matched donor (n=4); 1 patient underwent syngeneic transplantation from a twin sibling. Conditioning regimens included cyclophosphamide with either TBI (n=5), busulfan (n=2), or busulfan and alemtuzumab (n=1); and in one older patient, a reduced intensity conditioning (RIC) regimen consisting of busulfan, fludarabine and anti-thymocyte globulin was used. All patients achieved a complete remission; the patient who received bone marrow from his brother relapsed 19 months after transplant but was successfully re-transplanted with peripheral blood stem cells from this donor. Chronic graft-versus-host disease (GVHD) was observed in all allografted patients, and grade II-IV acute GVHD occurred in 5 of 8 patients (63%). The patient who received alemtuzumab developed cerebral toxoplasmosis and died of sepsis 273 days post transplant. A follow-up report by this group indicated that 21 patients with aCML had been transplanted, with 17 of 21 patients alive at 5 years after transplantation with a median survival of 47 months. These analyses compare more favorably to another study of allogeneic transplantation that included 7 patients with Ph chromosome-negative/BCR-ABL-negative CML. One patient suffered relapse at 9 months, and 5 of the patients had died by 3-26 months of follow-up.

A more recent retrospective study evaluating allogeneic HSCT in 10 MDS/MPN patients included 2 patients with aCML who received busulfan/ cyclophosphamide conditioning with bone marrow allografts from matched sibling donors. Both patients remained alive
with no evidence of disease after 96-99 months of follow-up. Notably, relapse was only observed in the 5 of 10 MDS/MPN patients who received RIC compared to none of the patients who received myeloablative conditioning. Because many patients with aCML or other MDS/MPN are elderly and may only be eligible for RIC, novel strategies are needed to reduce relapse in such individuals.

Molecular profiling to identify poor-risk mutations such as *SETBP1* and *ASXL1* may prompt earlier consideration of HSCT for eligible aCML patients. It is currently unknown whether HSCT can modify the adverse prognosis related to these mutations in the context of aCML. However, among 36 MDS/MPN patients with DNA available for serial molecular analysis, survival after HSCT was not influenced by *ASXL1, CBL, NRAS, or TET2* mutations (*SETBP1* was not assessed). Detection of these pre-transplant molecular markers, as well as *CSF3R T618I* may be useful for serial monitoring of minimal residual disease after HSCT, as their detection has been associated with overt relapse.

*Hypomethylating agents*

The use of hypomethylating agents in aCML is a rational application of their established activity in MDS and CMML. Among CMML patients treated in phase II studies of hypomethylating agents, overall response rates range from 25-70% (average 30-40%), with overall survival ranging from 12-37 months (reviewed in 38). It is challenging to extrapolate from the limited data on proliferative CMML patients to broadly inform how patients with aCML may respond. However, in one analysis, a WBC count > 13 x 10^9/L
and bone marrow blasts > 10% were adverse prognostic factors for response to azacitidine.  

I consider the use of azacitidine or decitabine in the following two scenarios: 1) as a bridging therapy for those who are eligible for HSCT; and 2) as stand-alone treatment for patients without a HSCT or clinical trial option. However, the experience with hypomethylating agents in aCML is limited and cannot be considered a standard of care. Decitabine (20 mg/m² daily intravenously x 5 days IV) produced a complete hematologic remission in 7 of the 8 patients described in four separate reports. Patients received a total number of 1 to 6 cycles; 4 patients achieved a complete hematologic remission (CHR) after 1 course of decitabine, and 3 patients achieved a CHR after 4 cycles. Response duration and length of follow-up was variably described in these publications; in one report, two patients were alive at 9 to 15 months after initiation of therapy, and 1 patient who maintained a response for 20 months expired at 26 months of follow-up. Two patients treated with decitabine were successfully bridged to transplant, with one succumbing to GVHD, BK viremia, and multiorgan failure on day +49. The small number of patients treated thus far precludes a determination of which clinical, laboratory, or molecular markers predict for response.

Ruxolitinib

Although uncommon, the identification of CSF3R T618I or JAK2 V617F in cases of aCML provides an opportunity to consider JAK inhibitor therapy since both of these
mutations result in JAK-STAT pathway activation. Pre-clinical studies indicate that ruxolitinib potently inhibits CSF3R T618I-driven malignant cell growth and can reduce leukocytosis and spleen size in a lethal myeloproliferative disease in mice driven by the mutation.44 However, such data do not supplant my recommendation to first consider HSCT for all eligible aCML patients, including individuals with either of these druggable mutations. JAK inhibition may useful to consider as a bridge to allogeneic HSCT; in the context of myelofibrosis, clinical improvement (vs. no clinical improvement) with JAK inhibitors (defined by International Working Group-Myeloproliferative Neoplasms Research and Treatment response criteria) was associated with improved overall survival after transplant in a multivariate analysis45 and may relate to improvement of performance status and reduction of splenomegaly.

Ruxolitinib is the only JAK inhibitor currently approved by the FDA (for patients with intermediate or high risk myelofibrosis, or for patients with polycythemia vera demonstrating intolerance or resistance to hydroxyurea). I recommend that patients be treated with this agent in the context of a clinical trial. Currently, a multicenter study (NCT02092324) is evaluating the safety and efficacy of ruxolitinib in patients with CNL and aCML, regardless of mutation status. However, if clinical trial enrollment is not feasible, I would consider off-label use of ruxolitinib in CSF3R T618I- or JAK2 V617F-mutated patients.

The potential benefit of ruxolitinib in CSF3R T618I-mutated disease was first demonstrated in a patient with CNL with CSF3R T618I who achieved a marked reduction
neutrophilic leukocytosis, and improvement of anemia and thrombocytopenia. Subsequently, a patient with hydroxyurea-refractory aCML dosed with ruxolitinib 10-20 mg twice daily resulted in similar hematologic improvements. Clinical benefits experienced by the patient included reduction of peripheral blood myeloid immaturity, marrow granulocytic hyperplasia and dysplastic megakaryocytes. In addition, ruxolitinib decreased in spleen volume by 75% after 3 months of therapy, reverted weight loss, and improved symptom scores. However, no change in CSF3R T618I mutant allele frequency was observed.

In the pediatric setting, ruxolitinib (50 mg/m²) has been used in an eleven year-old girl with aCML. Ruxolitinib decreased the leukocyte count from 101 to 7.9 x 10⁹/L after one week, ultimately permitting the patient to be bridged to a successful allogeneic HSCT. Although not well studied, the presence of additional mutations besides CSF3R T618I, such as SETBP1, may reduce responsiveness to JAK inhibitor therapy in aCML. Given its similar oncogenicity to CSF3R T618I in cellular transformation assays and in an in vivo murine transplantation model, aCML patients with the rarely described CSF3R T640N mutation would also be predicted to respond to JAK inhibition. As previously noted, patients with morphologic presentations consistent with aCML may exhibit rearrangements of JAK2, most notably PCM1-JAK2, which are sensitive to JAK inhibition, but with variable response duration. In contrast to CSF3R membrane proximal mutations, CSF3R truncation mutants preferentially activate the downstream Src family kinases and TNK2. Although in vitro assays showed that dasatinib could inhibit colony formation from bone marrow cells transduced with truncation mutant-
CSF3R-containing retroviruses, the efficacy of dasatinib in patients with these mutations has not yet been reported.

**Other Medical Therapies**

Complete and partial hematologic remissions have been reported with hydroxyurea in Ph chromosome/BCR-ABL1-negative CML patients, but the remissions are usually short-lived. Similar to its role in MPNs, I use hydroxyurea as a supportive care measure either alone, or as an adjunct to other therapies to control leukocytosis or progressive, symptomatic splenomegaly. Older studies evaluating standard interferon-alpha noted partial or complete hematologic remitting activity with variable durability of response. In a phase II study of PEG-interferon-a-2b (starting dose of 3 mcg/kg/week), 2 of 5 BCR-ABL1 negative CML patients achieved complete remission after 3 months of therapy. Median duration of therapy was 36 and 38 months at which time both patients were discontinued due to toxicity. Given the more favorable toxicity profile of pegylated interferons, these extended formulations merit further investigation in aCML. Because treatment of anemia remains an unmet need, it may also be fruitful to explore whether factors predicting response to erythropoiesis stimulating agents in MDS also have potential applicability to aCML.

Splenectomy and splenic irradiation have limited roles in the management of aCML. In rare circumstances, either therapeutic modality may be useful for disease palliation when other options have failed. However, the use of either modality must be weighed
against morbid complications such as bleeding, thrombosis, infection, and potential for acceleration of leukocytosis and hepatomegaly.

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Return to the Cases

Based on the limited data available for aCML therapy, and the diagnostic and treatment algorithms I have outlined, we now return to the disposition of the two cases.

Case 1

We obtained a myeloid mutation panel on patient LJ which revealed three pathogenic variants: \textit{SETBP1} G870S (mutant allele frequency 45\%), \textit{SRSF2} P95H (mutant allele frequency 50\%), and \textit{ASXL1} P808fs*10 (1 base pair deletion with frame shift; mutant allele frequency 45\%). Although the patient’s white blood cell count was only mildly elevated and the hemoglobin and platelets were well preserved, we discussed our concern about aCML-associated survival as well as her molecular profile—specifically the unfavorable prognosis associated with \textit{SETBP1}, and the generally poor-risk related to \textit{ASXL1} and \textit{SRSF2} in the context of other myeloid neoplasms. The patient underwent consultation for a reduced-intensity conditioning HSCT and for HLA typing. Because her 2 siblings were not matches, an unrelated donor search was initiated. Over the next 2 months, her WBC count increased to 48 x 10^9/L, and progressive cytopenias developed (Hb 9.5 g/dL; platelets 85 x 10^9/L). A repeat bone marrow revealed increased blasts (8\%) without clonal cytogenetic evolution. We recommended decitabine therapy (20 mg/m^2 IV x 5 days on 28-day cycles). After 3 cycles, she achieved a complete hematologic remission and a repeat bone marrow showed 3\% blasts with persistent
trilineage dysplasia. A 10/10 unrelated donor was identified and the patient proceeded to a RIC HSCT. She remains in a hematologic and molecular remission 15 months after transplant with mild-moderate chronic GVHD.

Case 2

This patient’s aCML was likely masked by treatment of his hepatitis C with PEG-IFN-alpha-2a. With stoppage of treatment, laboratory features of aCML emerged. Due to CK’s age and performance status, a mutual decision was made by the patient and physician to seek alternative treatment besides HSCT. Because he carried the CSF3R T618I mutation (albeit uncommon in aCML), he decided to pursue a clinical trial with ruxolitinib. In order to be eligible, he first underwent treatment with ledipasvir/sofobuvir which eradicated his hepatitis C. Before trial initiation, his CBC revealed a WBC count of 32.7 x 10⁹/L, Hb 9.5 g/dL, and platelet count of 57 x 10⁹/L, and the spleen had increased to 7 cm below the left costal margin. No significant changes in the bone marrow were observed except a borderline increase in blasts (5%). Treatment with 4 months of ruxolitinib in the range of 5-10 mg twice daily eliminated splenomegaly and markedly improved his symptom burden. In addition, the WBC count decreased to 11.9 x 10⁹/L, and the platelet count improved to 130 x 10⁹/L; however, the patient became red blood cell transfusion-dependent. After 2 additional months of therapy, the WBC and platelet count began to worsen again. A repeat marrow demonstrated a further increase in blasts to 10%. The patient has recently been switched to decitabine therapy.
Future Prospects

In this era of precision medicine, it is incumbent on physicians evaluating aCML patients to employ myeloid mutation panels to uncover potentially druggable targets. A recent example comes from investigators from Oregon Health and Sciences University who identified an NRAS G12D mutation at 47% mutant allele frequency in an aCML patient.\(^5^7\)

The mitogen-activated protein kinase kinase 1 (MEK1)/MEK2 inhibitor trametinib, approved for malignant melanoma, also exhibits activity in RAS-driven leukemias \textit{in vitro} and \textit{in vivo}.\(^5^8,^5^9\) Treatment with trametinib 2 mg daily produced a durable near-complete hematologic response, with the WBC count decreasing from 256 x 10\(^9\)/L to the 10 to 15 x 10\(^9\)/L range and the platelet count improving from 66 to 168 x 10\(^9\)/L.\(^5^7\)

Pharmacologic reactivation of the tumor suppressor PP2A, which is functionally suppressed due to \textit{SETBP1} mutations, is a promising therapeutic approach to pursue with drugs such as fingolimod (FTY720).\(^6^0,^6^1\) Spliceosome modulators are a novel class of therapeutics entering clinical trials for myeloid neoplasms and may have a role in aCML patients with mutations in \textit{SRSF2} or other genes that comprise the spliceosome machinery.\(^6^2\) Combination strategies employing therapies targeting disease-associated mutations in conjunction with either 1) hypomethylating agents, or 2) as an adjunct to HSCT (either as a bridge to transplant or in the post-transplant setting to reduce relapse) should be evaluated. Lastly, I encourage use of the new international MDS/MPN response criteria so that treatment responses between regimens can be more accurately compared.\(^6^3\)
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inhibition in a patient with CSF3R-T618I-positive atypical chronic myeloid

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159.

leukemia with concomitant CSF3R T618I and SETBP1 mutations


Table 1. World Health Organization Diagnostic Criteria for Atypical Chronic Myeloid Leukemia, BCR-ABL1-negative

- Peripheral blood leukocytosis (WBC ≥ 13x10^9/L) due to increased numbers of neutrophils and their precursors with prominent dysgranulopoiesis
- Neutrophil precursors (promyelocytes, myelocytes, metamyelocytes) ≥ 10% of leukocytes
- No Ph chromosome or BCR-ABL1 fusion gene and not meeting criteria for PV, ET, or PMF*
- No evidence of PDGFRA, PDGFRB, FGFR1 rearrangement, or PCM1-JAK2
- Minimal absolute basophilia; basophils usually <2% of leukocytes
- No or minimal absolute monocytosis; monocytes usually < 10% of leukocytes
- Hypercellular bone marrow with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
- Less than 20% blasts in the blood and bone marrow

PV: polycythemia vera; ET: essential thrombocytemia; PMF: primary myelofibrosis

* Cases of MPN, particularly those in accelerated phase and/or in post-polycythemic or post-essential thrombocytemic myelofibrosis, if neutrophilic, may simulate aCML. A previous history of MPN, the presence of MPN features in the BM and/or MPN-associated mutations (in JAK2, CALR, or MPL) tend to exclude a diagnosis of aCML. Conversely, a diagnosis of aCML is supported by the presence of SETBP1 and/or ETNK1 mutations. The presence of a CSF3R mutation is uncommon in aCML and if detected should prompt a careful morphologic review to exclude an alternative diagnosis of CNL or other myeloid neoplasm.1
Figure Legends

Figure 1. Diagnostic evaluation for atypical CML and identification of opportunities for targeted therapy. If a morphologic diagnosis of aCML is rendered, cytogenetic, FISH, and myeloid mutation panel testing are critical as they can unmask karyotypic or molecular abnormalities that have potential implications for use of targeted therapy approaches.

*The ability to target certain genes is expected to change over time as new therapeutics are developed

§ Additional JAK2 rearrangements besides the PCM1-JAK2 fusion may present with morphologic features of aCML

Figure 2. Treatment algorithm for atypical CML. Please refer to the text section ‘Treatment’ for a discussion of this treatment scheme for aCML. This algorithm is based on several decision nodes, including: 1) potential candidacy for allogeneic hematopoietic stem cell transplantation (HSCT); 2) the results of myeloid mutation panel testing; 3) eligibility for enrollment in clinical trials; and 4) opportunities to adopt strategies used for MDS or MPN (e.g. hypomethylating agents or second line/adjunctive therapies).

HSCT: hematopoietic stem cell transplantation; ESAs: erythropoiesis-stimulating agents.

*The ability to target certain genes is expected to change over time as new therapeutics are developed

** Myeloid mutation panel testing may also be performed prior to patients proceeding directing to allogeneic HSCT who do not require pre-transplant disease cyto reduction.
CBC with differential
Bone marrow aspirate and biopsy
- Morphologic review (PB and BM)
  - PB leukocytosis and % neutrophil precursors
  - Assess for dysplastic granulopoiesis + erythroid/megakaryocytic dysplasia
  - Percent blasts
  - Absolute and percent basophilia & monocytosis
- Standard cytogenetics/FISH
- PCR for BCR-ABL1
- Myeloid mutation panel testing

Ph positive or BCR-ABL1 positive

Chronic myeloid leukemia

Morphology consistent with aCML and BCR-ABL1 negative

Cytogenetics/FISH Analysis

HLA typing

Myeloid mutation panel testing

Cytogenetics, FISH, and/or PCR evidence for rearrangements involving PDGFRα (4q12), PDGFRβ (5q31-33), FGFR1 (8p11), or JAK2 (9p24)$

Non-specific karyotype abnormalities (e.g. trisomy 8, del(20q), -7/7q-, isochromosome 17q)

Cases reassigned from aCML to the WHO category of 'Myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of PDGFRα, PDGFRβ, FGFR1, or PCM1-JAK2'; targeted therapy or other treatment options based on specific rearrangement

Potentially actionable myeloid gene mutations* (e.g. JAK2, CSF3R, N/K-RAS)

Myeloid mutations not currently actionable* (e.g. SETBP1, ETNK1, ASXL1, TET2, etc.)

See Figure 2 for treatment algorithm and considerations for targeted therapy
Diagnosis of aCML

HLA typing

Candidate for allogeneic HSCT?

Myeloid mutation panel testing

Potentially actionable myeloid mutation(s)*

Consider therapy with targeted agent (e.g. JAK inhibitor for JAK2 or CSF3R mutation; or MEK inhibitor for RAS mutation) on a clinical trial (preferred) or off-label basis

Hypomethylating therapy (decitabine or azacitidine); second-line or adjunctive options include: PEG-interferon-α; hydroxyurea, ESAs

No currently actionable myeloid mutation(s)*

Clinical trial not based on an actionable mutation

Is pre-transplant disease cytoreduction required during HLA typing of potential donors?

no

Proceed to allogeneic HSCT**

yes

yes

no

Figure 2.
How I treat atypical chronic myeloid leukemia

Jason Gotlib