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Leukemia (PH Wiernik, Section Editor)

Current Management of Chronic Neutrophilic Leukemia

Natasha Szuber, MD[1,*]
Ayalew Tefferi, MD[2,*]

Address
[1]Department of Internal Medicine, Division of Hematology, Maisonneuve-Rosemont Hospital, Montreal, 5415 Blvd. del'Assomption, Montreal, Quebec, H1T 2M4, Canada
[*,2]Department of Internal Medicine, Division of Hematology, Mayo Clinic, 200 First St. SW, Rochester, MN, 55905, USA
Email: natasha.szuber@umontreal.ca
Email: tefferi.ayalew@mayo.edu

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Opinion statement

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative neoplasm (MPN) characterized by oncogenic driver mutations in colony-stimulating factor 3 receptor (*CSF3R*). Due in large part to the rarity of the disease and dearth of clinical trials, there is currently no standard of care for CNL. Available therapies range from conventional oral chemotherapy to targeted JAK inhibitors to hematopoietic stem cell transplant (HSCT), the latter representing the only potentially curative modality. For this reason, coupled with CNL's typically aggressive clinical course, allogeneic HSCT remains the primary recommended therapy for eligible patients. For ineligible patients, a number of nontransplant therapies have been evaluated in limited trials. These agents may additionally be considered "bridging" therapies pre-transplant in order to control myeloproliferation and alleviate symptoms. Historically, the most commonly utilized first-line agent has been hydroxyurea, though most patients ultimately require second (or subsequent)-line therapy; still hydroxyurea remains the conventional frontline option. Dasatinib has demonstrated efficacy in vitro in cases of *CSF3R* terminal membrane truncation mutations and may cautiously be considered upfront in such instances, though no substantive studies have validated its efficacy in vivo. Numerous other chemotherapy agents, practically re-appropriated from the pharmaceutical arsenal of MPN, have been utilized in CNL and are typically reserved for second/subsequent-line settings; these include interferon-alpha (IFN-a), hypomethylating agents, thalidomide, cladribine, and imatinib, among others. Most recently, ruxolitinib, a JAK1/2 inhibitor targeting JAK-STAT signaling downstream from *CSF3R*, has

emerged as a potentially promising new candidate for the treatment of CNL. Increasingly robust data support the clinical efficacy, with associated variable reductions in allele burden, and tolerability of ruxolitinib in patients with CNL, particularly those carrying the *CSF3R*T618I mutation. Similar to conventional nontransplant strategies, however, no disease-modifying or survival benefits have been demonstrated. While responses to JAK-STAT inhibition in CNL have not been uniform, data are sufficient to recommend consideration of ruxolitinib in the therapeutic repertory of CNL. There remains a major unmet need for prospective trials with investigational therapies in CNL.

Introduction

Chronic neutrophilic leukemia (CNL) is a rare *BCR-ABL1*-negative myeloproliferative neoplasm (MPN) characterized by sustained mature neutrophilic leukocytosis, bone marrow granulocyte hyperplasia, and frequent hepatosplenomegaly. In 2013, a landmark study identifying oncogenic driver mutations in colony-stimulating factor 3 receptor (*CSF3R*) in the majority (89%) of patients with CNL established a critical clinico-molecular nexus defining the disease [1]. The *CSF3R* mutation further serves as a biomarker for diagnosis and provides, along with associated downstream pathways, a putative target for pharmacological intervention. The revised World Health Organization (WHO) criteria formally integrated *CSF3R* mutations as a central diagnostic marker for CNL in 2016 [2] along with previously established criteria as follows: leukocytosis $\geq 25 \times 10^9$/L with $\geq$ 80% neutrophils/band forms and < 10% neutrophil precursors, monocyte count $< 1 \times 10^9$/L, and absence of dysgranulopoiesis, as well as hypercellular bone marrow with granulocyte hyperplasia, normal maturation and < 5% myeloblasts, and absence of fulfillment of criteria for other MPN. The 2016 WHO diagnostic criteria for CNL are summarized in Table 1. CNL remains a rare entity, underscored by a recent population-based study which found an overall incidence of 0.1 cases/1,000,000 individuals using combined Surveillance, Epidemiology, and End Results (SEER) and National Cancer Database (NCDB) data [3•].

From a clinical perspective, CNL is excessively heterogeneous. While commonly presenting as incidental neutrophilic leukocytosis in asymptomatic subjects, a wide spectrum of manifestations may be observed, including constitutional symptoms, bone pain, pruritus, and symptoms related to splenomegaly and/or hepatomegaly [4, 5] as well as bleeding diathesis, with propensity towards cerebral hemorrhage [6–9]. The overall prognosis is variable, but generally unfavorable with the latest reported median overall survival of 1.8 years (95% CI: 1.3–2.5) [3•]. Soberingly, this same study revealed no improvement in survival in contemporary cohorts versus historical ones, emphasizing the patent need for more efficacious, disease-altering therapies.

An operational prognostic scoring system was recently developed for CNL establishing platelet count $< 160 \times 10^9$/L (2 adverse points), leukocyte count $> 60 \times 10^9$/L (1 point), and presence of an *ASXL1* mutation (1 point) as independent adverse prognostic variables [10•]. Patients allocated to low-risk (0–1 points) or high-risk (2–4 points) categories had respective overall survival times that were "not yet reached" vs 22.4 months (p=0.0016) [10•].

There is currently no standard of care for the management of CNL, reflecting the rarity of the disease, dispersion of patients, limited survival rates, and appreciable barriers to conducting adequate clinical trials in this population. Management approaches to CNL include watchful waiting, splenic irradiation, oral chemotherapy, interferon-alpha, kinase inhibitors, JAK inhibitors, and hematopoietic stem cell transplant (HSCT). A large institutional case series we published disclosed frontline therapy most frequently consisting of hydroxyurea, followed by interferon-alpha, then a miscellany of other agents including thalidomide, tyrosine kinase inhibitors (imatinib, dasatinib), cladribine, and azacitidine. Second-line therapy most often consisted of ruxolitinib, stem cell transplant, or other agents as mentioned [10•]. When deciding upon initial therapy, other than *CSF3R* mutation subtype (membrane

Table 1. World Health Organization 2016 diagnostic criteria for chronic neutrophilic leukemia. Adapted from [2]

1. Peripheral blood WBC ≥ 25 × 10^9/L
- Segmented neutrophils plus band forms ≥ 80% of WBC
- Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) < 10% of WBC
- Myeloblasts rarely observed
- Monocyte count < 1 × 10^9/L
- No dysgranulopoiesis
2. Hypercellular bone marrow
- Neutrophil granulocytes increased in percentage and number
- Normal neutrophil maturation
- Myeloblasts < 5% of nucleated cells
3. Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, ET, or PMF
4. No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or *PCM1-JAK2*
5. Presence of *CSF3R*T618I or other activating *CSF3R* mutations
or
In the absence of a *CSFR3R* mutation, persistent neutrophilia (at least 3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies

WBC, white blood cells; *CML*, chronic myeloid leukemia; *PV*, polycythemia vera; *ET*, essential thrombocythemia; *PMF*, primary myelofibrosis

proximal vs truncation), discussed below, there is insufficient data to recommend one agent above another and the choice ultimately relies on physician experience, physician/patient preference, and clinical context, including attentiveness to mutation profile and reported drug resistance. While HSCT remains the only curative therapy, there are limited accounts to guide us in determining *when* and *in whom* to endorse this approach [11•]. Given the generally guarded prognosis of CNL, however, a reasonable guiding principle may be to consider HSCT as the premier therapy in eligible patients with adequate donor options.

Though therapeutic decision-making remains challenging, novel data on molecular prognostic markers and the genetic basis of clonal evolution and drug resistance, as well as the realization of more robust clinical trials in CNL, such as a recent phase II study of JAK inhibitors [12••], are informing and refining state-of-the-art management in CNL.

Molecular pathogenesis and genomics: the new foundation of therapeutics in CNL

Upon binding to the *CSF3R* receptor, granulocyte colony-stimulating factor (G-CSF) exerts its effect through activation of classical downstream pathways Janus kinase (JAK)-signal transducer and activator of transcription (STAT), SRC family kinases (including LYN) [13, 14], and Ras/Raf/MAP kinases, among others [15]. Two prototypical mutational variants of *CSF3R* have been identified: (i) membrane proximal (more common, primarily T618I and T615A point mutations); and (ii) frameshift or nonsense mutations leading to premature truncation of

the *CSF3R* cytoplasmic tail (e.g., D771fs, S783fs, Y752X, and W791Z) [1, 16]. Mechanistically, membrane proximal mutations result in constitutive ligand-independent receptor activation and downstream signaling through JAK-STAT [1, 17], while truncation lesions produce a loss of negative regulatory motifs, disrupting receptor trafficking and delaying receptor internalization and/or degradation [16, 18], thereby increasing *CSF3R* cell surface expression [19]. Importantly from a therapeutic standpoint, Maxson et al.'s seminal 2013 report disclosed differential susceptibilities of the two *CSF3R* mutation types to tyrosine kinase inhibitors, with membrane proximal and truncation mutations preferentially responding to the JAK inhibitor ruxolitinib and SRC kinase inhibitor dasatinib, respectively [1]. This data corroborates the critical concept of molecularly directed drug targeting in CNL.

Genotype-phenotype associations have also been demonstrated in CNL. Subdividing *CSF3R*-mutated CNL patients into "*CSF3R*T618I" versus "other" *CSF3R* mutations, our group recently exposed two phenotypically and prognostically distinct subsets, with individuals harboring the classic *CSF3R*T618I lesion exhibiting adverse clinical characteristics including inferior overall survival, compared to their *CSF3R*T618I-unmutated counterparts [10•]. High-precision genetic profiling has illuminated an increasingly complex genomic landscape in CNL involving not only *CSF3R* but additional prognostically relevant mutations. Concurrent mutations occur with variable but often substantial frequencies and include epigenetic components, *SETBP1*, and spliceosome complex, as well as signaling mutations [5, 20–25].

An elegant 2019 study by Zhang et al. reported on genomic and transcriptomic profiling in CNL (n = 39) as well as other neutrophilic leukemias [26••]. The most frequently mutated genetic pathways involved chromatin modification (*ASXL1*, 65.8%; *EZH2*, 19%; and *ASXL2*, 3.2%), DNA modification (*TET2*, 33.5% and *DNMT3A*, 5.7%), and spliceosome complex members (*SRSF2*, *U2AF1*, *SF3B1*, *ZRSR2*, and *RPRF8*) with a total incidence of 55.7% [26••]. A high level of genetic complexity was manifested by a high median number of mutations per patient (3.6; range, 0–8) [26••]. Notably, drug sensitivity assays were performed in cells with concomitant *CSF3R* and *NRAS* mutations, disclosing reduced sensitivity to either ruxolitinib or trametinib, but response to the combination of drugs.

There are important therapeutic implications as the scope of our understanding of the genetic landscape of CNL broadens. Evidence that recurrent disease-modifying mutations (e.g., *ASXL1*, *SETBP1*, spliceosome genes) confer a poor prognosis [27] and identification of markers of clonal evolution over the disease course [28••] not only provide additional insight into disease biology and progression, but also potentially translate to novel drug targets and treatment strategies.

Treatment options

There are currently no evidence-based guidelines for the management of CNL. With the exception of hematopoietic stem cell transplant, often limited to a minority of eligible patients, no pharmacological agents have been shown to significantly alter disease progression or ameliorate survival. The development

of potentially disease-modifying therapies thus remains a major unmet need. Treatment options consist of (i) pharmacological agents (hydroxyurea, interferon, kinase inhibitors, and JAK inhibitors); (ii) interventional procedures (primarily splenic irradiation and allogeneic hematopoietic stem cell transplant); and (iii) investigational therapies.

Approach to management

Once CNL diagnosis is confirmed, performing gene panel-based screening for *ASXL1* and *SETBP1* mutations in both *CSF3R*-mutated and unmutated CNL patients could be advocated in light of these mutations' potential prognostic significance. Risk stratification according to our institution's model [10•] detailed above may be useful in broadly discriminating between low- versus high-risk individuals. Eligible patients should be assessed for the possibility of allogeneic HSCT. All patients, regardless of disposition for and/or prospective transplant plans, should undergo careful clinical evaluation and pharmacologic therapy initiated for either uncontrolled myeloproliferation (a reasonable target being leukocyte count < 25–30 $\times 10^9$/L) or associated symptoms. A proposed algorithm for management of CNL is provided in Fig. 1.

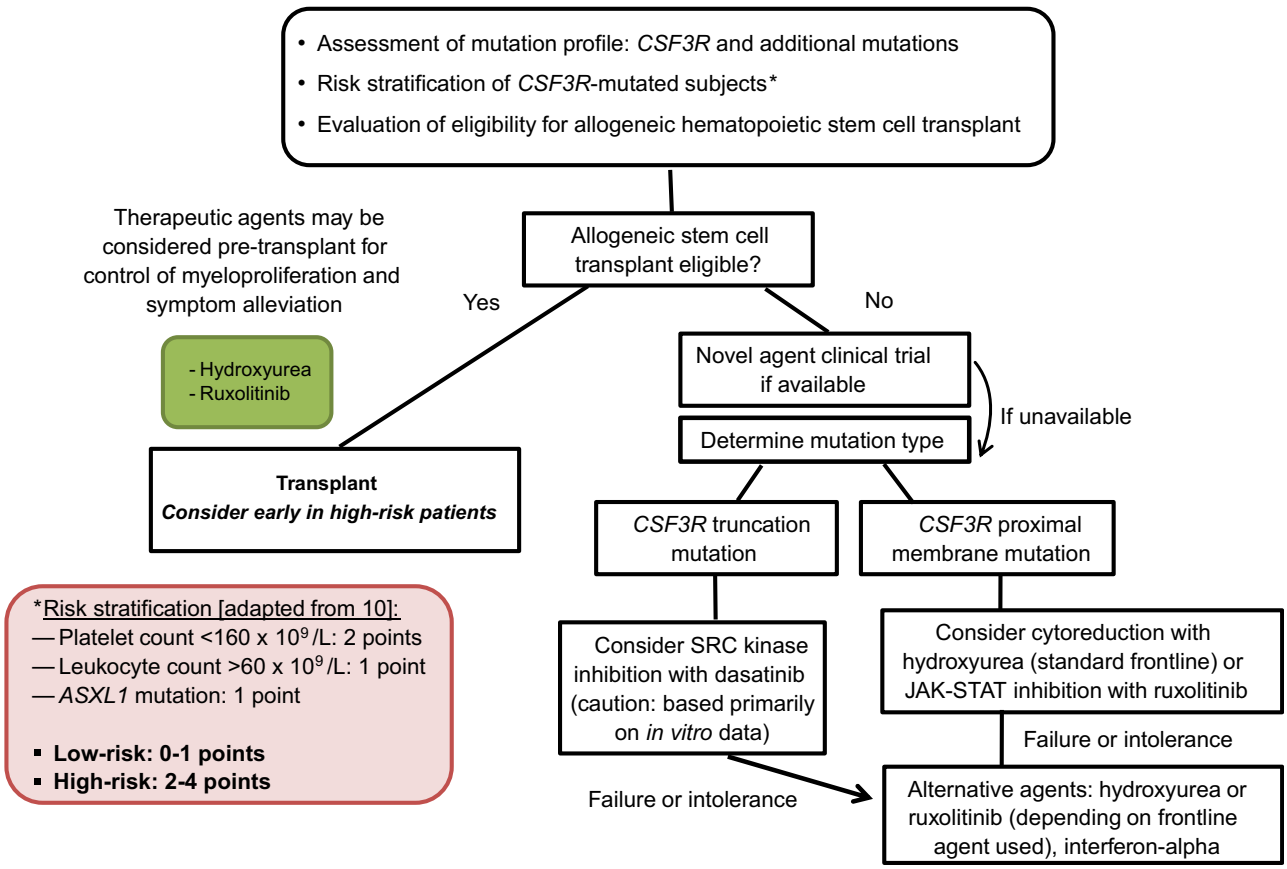

Fig. 1. Proposed algorithm for management of chronic neutrophilic leukemia.

Pharmacologic

Pharmacologic agents are not curative, nor do they prolong survival in CNL. The treatment aims of drug therapy are thus exclusively mitigatory and include stabilization of myeloproliferation/leukocytosis, symptom palliation and reduction of splenomegaly, and improvement of quality of life. Treatment options in this category include conventional oral chemotherapy with hydroxyurea, immune modulation with interferon, and targeted therapy with kinase inhibitors and, most recently, ruxolitinib. A summary of the most commonly used pharmacologic agents in CNL is presented in Table 2.

Conventional chemotherapy/targeted therapy

Hydroxyurea

- Standard dosage: oral 500–2000 mg daily; requires dose titration.
- Contraindications: hypersensitivity, severe cytopenias.
- Main side effects: dermatological, gastrointestinal, cytopenias, macrocytosis, propensity to infection.
- Cost-effectiveness: inexpensive.
- Special points:

Oral chemotherapy has been documented as the backbone of CNL management since the 1970s [4, 30, 31]. Hydroxyurea has been the most commonly utilized agent in CNL with up to 75% of patients showing an initial response (reduction in leukocytosis and/or splenomegaly) with median duration of therapy of 12 months (range: 6–87) [29]. In our institution's study of 19 molecularly annotated CNL patients, the vast majority (82%) were treated with hydroxyurea as a first-line agent and it was ultimately received by all (100%) over the disease course [10•]. However, the majority required second-line therapy (53%) and nearly a third required three lines of treatment or more (32%). Notably, up to 25% of patients were non-responsive to hydroxyurea therapy from the outset [29], though no predictive markers of sensitivity have been identified. A recent study by Dao et al. documented upfront hydroxyurea therapy in 81.5% of patients who subsequently went on to receive ruxolitinib [12••], confirming its routine use as a frontline agent. As there are currently no controlled studies favoring the use of drugs other than hydroxyurea as first-line therapy in CNL, this remains the convention.

Immune modulation

Interferon-alpha

- Standard dosage: subcutaneous 0.5 to 1 million units 3 times/week, titrating up to 2–3 million units 3 times/week.
- Contraindications: hypersensitivity, active liver disease, autoimmune hepatitis.
- Main side effects: flu-like symptoms (fatigue, headache, chills, myalgias/arthralgias, fever), depression, dizziness, dermatologic (alopecia, rash), weight loss/anorexia, gastrointestinal (nausea/vomiting), cytopenias,

Table 2. List of most common currently available pharmacologic therapies for chronic neutrophilic leukemia

Pharmacologic agent	Most frequently reported indication	Route	Standard dose	Reported response rates	Main side-effects	Key references
Hydroxyurea	Frontline	Oral	500–2000 mg daily; requires dose titration.	- Initial response rate up to 75% in case series [10•]. - Median duration of therapy of 12 months (range: 6–87) [29].	Dermatological, gastrointestinal, cytopenias, macrocytosis, propensity to infection.	[4, 10•, 12••, 29–31].
Interferon-alpha	Second-line or subse-quent	Subcutaneous	0.5 to 1 million units 3 times per week, titrating up to 2–3 million units 3 times per week.	- Too few informative studies to derive response rates. - Durable remissions documented in limited case reports [5, 9, 32, 33].	Flu-like symptoms, depression, dizziness, dermatologic (alopecia, rash), weight loss/anorexia, nausea/vomiting, cytopenias, increased liver enzymes, injection site reactions.	[5, 9, 32, 33].
Ruxolitinib	Second-line or subse-quent	Oral	5–20 mg twice daily. Product monograph recommends dose adjustment according to platelet count.	- Phase II trial: 35% response rate (4 CR, 9 PR); 85% met criteria for at least 1 category of clinical benefit [12••]. - Presence of *CSF3R* mutations correlate with response. - Reported decrease in *CSF3R* allele burden [12, 28, 34, 35]. - Successful reported use as "bridge" to transplant [10•].	Dermatological, increased cholesterol and triglyceride levels, diarrhea, cytopenias, increased liver enzymes, headache/dizziness. Requires dose taper when discontinuing.	[1, 10•, 12••, 34–40].

CR, complete response; *PR*, partial response

increased liver enzymes, injection site reaction.
- Cost-effectiveness: inexpensive
- Special points:

Interferon-alpha also has a long history of use in CNL and is the only agent with which durable remissions have been documented in limited case reports [5, 9, 32, 33]. Meyer et al. described two CNL patients with progressive disease treated with IFN-a [32], both eventually achieving long-term disease stabilization after treatment durations of 16 and 26 months respectively. One patient experienced slow progression after discontinuing IFN, though never required re-treatment after a follow-up period of 90 months, while the second had stable disease (follow-up period 66 months). Four additional studies similarly report long-term hematological and clinical remissions with IFN-a [5, 9, 33] or pegylated interferon [41] in CNL lasting from 24 to 41+ months. IFN-a, thus, not only is a safe and effective option for CNL but also offers the potential for durable remissions.

While benefits of IFN must be counterbalanced by its potentially serious side effects, there is sufficient rationale to maintain its inclusion in our therapeutic arsenal for CNL, particularly for patients of childbearing age or as a second- or subsequent-line agent failing frontline therapy.

Kinase inhibitors

Dasatinib

- Standard dosage: oral 100 mg daily.
- Contraindications: hypersensitivity.
- Main side effects: edema/fluid retention, headache, fatigue, rash, diarrhea, cytopenias, infection risk, musculoskeletal pain, pleural effusion.
- Cost-effectiveness: moderately expensive.
- Special points:

The first justification for the use of the SRC kinase inhibitor dasatinib in the management of CNL came from Maxson et al.'s seminal 2013 report in which *CSF3R* truncation mutations, documented to operate predominantly through SRC kinases, exhibited in vitro drug sensitivity to dasatinib [1]. Scarce literature exists, however, on dasatinib activity in CNL patients in vivo. Interestingly, a recent study documented a favorable response to chemotherapy *plus* dasatinib in a patient with B-cell acute lymphoblastic leukemia harboring a *CSF3R* truncation mutation [42]. However, it is speculated that in the context of additional proximal membrane mutations, which frequently co-occur in CNL, dasatinib may not be sufficient [36]. Hinze et al. reported on a compound *CSF3R*-mutated CNL patient (T618I and Q749X mutations) refractory to dasatinib, who ultimately required ruxolitinib treatment as salvage [36], challenging dasatinib's efficacy in vivo, particularly in cases of concurrent truncation and proximal mutations.

Thus, with few studies (based primarily on in vitro evidence) to support its use, and concern for lack of efficacy in compound mutants, use of dasatinib in CNL remains provisional and somewhat exploratory. While a short trial may be reasonable in CNL patients with isolated cytoplasmic tail truncation mutations, close monitoring for lack of response is recommended.

JAK inhibitors

Ruxolitinib

- Standard dosage: oral 5–20 mg twice daily (product monograph recommends dose adjustment according to platelet count).
- Contraindications: hypersensitivity, current or previous history of progressive multifocal leukoencephalopathy.
- Main side effects: dermatological, increased cholesterol/triglyceride levels, diarrhea, cytopenias, increased liver enzymes, headache/dizziness. Requires dose taper when discontinuing.
- Cost-effectiveness: expensive.
- Special points:

The JAK1/2 inhibitor ruxolitinib is approved for treatment of patients with intermediate- or high-risk myelofibrosis and polycythemia vera intolerant/refractory to hydroxyurea [37, 38]. Though not FDA-approved for CNL, ruxolitinib has been assessed both in murine models and individuals with *CSF3R*-mutated CNL and atypical chronic myeloid leukemia (aCML). The first account of ruxolitinib use in CNL patients stems from Maxson et al.'s 2013 study [1], wherein ruxolitinib induced a clinical response in a *CSF3R*T618I-mutated patient which was maintained after 11 months of therapy [39]. Ruxolitinib efficacy was subsequently validated in a *CSF3R*T618I+ mouse model [40]. A larger series of 19 CNL patients from our institution disclosed variable responses to ruxolitinib received either as second-line (n = 3) or third-line (n = 1) therapy, with all patients having previously been exposed to hydroxyurea [10•]. One case showed a favorable response, two cases experienced initial response but eventually progressed (response durations ~ 9.5 and 36 months, respectively), and one case receiving ruxolitinib in blast phase as a "bridge" to transplant (duration of therapy ~ 0.5 months) ultimately had a favorable outcome and was alive at last follow-up ~ 46 months from diagnosis [10•].

A much-anticipated phase II clinical trial assessing the safety and efficacy of ruxolitinib in patients with CNL (n = 21) and aCML (n= 23) was recently published [12••]. Overall response rate was 35%, including 4 complete and 9 partial responses in the CNL cohort and 85% of patients met the criteria for at least 1 category of clinical benefit(s). Diagnosis of CNL (as opposed to aCML) and presence of *CSF3R* mutations strongly correlated with response to ruxolitinib, defined by control of leukocytosis and spleen volume reduction. Median survival was higher in responders versus non-responders (23.1 vs 15.6 months). Two CNL patients with complete responses lasting > 1 year (and ongoing) presented with lower risk features, suggesting that ruxolitinib may be more effective early in disease pathogenesis; however, additional studies are required to evaluate the impact of JAK inhibitor therapy on natural history and clonal evolution in CNL. Interestingly, the range of responses to ruxolitinib is speculated to be at least partially due to co-occurring mutations, as will be further discussed.

Greater complexity in assessing the efficacy of ruxolitinib in CNL emerges when considering the modulation of response according to mutational spectrum. Studies specifically evaluating response to ruxolitinib in CNL subjects co-

expressing *CSF3R* and *SETBP1* mutations, a reportedly prognostically detrimental combination [20], have yielded inconsistent results [34, 43, 44]. Furthermore, compound *CSF3R* mutants (co-existence of proximal and truncation mutations) have demonstrated resistance to ruxolitinib in murine models [45], while conversely, a recent case report of Hinze et al. showed durable hematologic remission lasting > 3 years following diagnosis following ruxolitinib therapy in a 71-year-old man with compound *CSF3R* mutations (T618I and Q749X) previously treated with and failing dasatinib [36].

A very recent study by Stoner et al. provided much-needed insights into clonal evolution in CNL patients on ruxolitinib therapy [28••]. Salient findings included (i) detection of *STAT3* mutations in patients later in ruxolitinib treatment course, projecting this as a key mechanism of ruxolitinib resistance; and (ii) detection of *RUNX1* and *STAG2* mutations at disease progression ($n = 3$ each). The latter observation suggests a role for cooperating *RUNX1* and *CSF3R* mutations in CNL progression and as an early marker of AML transformation, as well as a role for *STAG2* mutations as a late indicator of disease progression in CNL patients on ruxolitinib. Additionally, *CBL* mutations were identified in four patients with mutant clones displaying variable responses to ruxolitinib. The authors correspondingly suggest that monitoring *CBL* mutations may be helpful in tracking drug response and disease progression. This is notably the first study to assess changes in clonal evolution in CNL patients over the course of ruxolitinib treatment. Indeed, mutational variants that emerge per ruxolitinib regime may predict drug resistance, though details of the associated underlying mechanisms need to be elucidated. Implications for future management strategies are primordial, though, as additional molecular culprits, may need to be targeted (e.g., *STAT3*, *RUNX1*) in order to overcome resistance-related pathways and provide synergistic elimination of malignant clones/sub-clones to effectively impede disease progression.

The impact of JAK inhibitor therapy on *CSF3R* allele burden has been evaluated in multiple studies with conflicting results. Dao et al. found no effect [46], while Nooruddin et al. and Gunawan et al. observed reductions in allele burden in CNL cohorts treated with ruxolitinib, though no systematic correlations with symptom improvement were found [34, 35]. Variable responses were also found in Stoner et al.'s report, with 3 patients presenting allele burden reductions while 2 showed minimal change over time [28••]. Similarly, mixed results were found in Dao et al.'s phase II trial of ruxolitinib in 21 patients with CNL [12••].

Though additional studies are required to further determine the impact of JAK inhibitor therapy on natural history in CNL, available data are encouraging enough to establish ruxolitinib as a therapeutic resource in CNL. Practical considerations which have yet to be addressed include timing of therapy initiation, use as frontline versus subsequent-line agent, and pre-transplant administration in HSCT-eligible patients. Our approach to these issues is as follows:

i) While some data support initiation early in the disease course, prior to development of severe symptoms or cytopenias, and acquisition of mutant sub-clones [12••], these require further validation and we currently would not endorse empirical ruxolitinib use unless indicated for control of myeloproliferation and/or symptom management.

ii) As most reports on ruxolitinib therapy in CNL involve its use as a second-line agent [10, 12], we recommend therapy initiation with hydroxyurea and alternative use of ruxolitinib should lack of response and/or intolerance develop [47]. Albeit frontline ruxolitinib therapy may be justified in certain circumstances (e.g., heavy symptom burden/massive splenomegaly, severe cytopenias for which hydroxyurea may be prohibitive), at the discretion of the treating physician.

iii) Although no studies have specifically assessed ruxolitinib use peri-transplant in CNL, preliminary data [10•] and extrapolation from experience in other MPN such as myelofibrosis [48, 49] suggest this may be an acceptable approach, particularly when patients require immediate treatment for leukocytosis or symptom control.

Induction chemotherapy

Standard induction "7 + 3" chemotherapy has not been demonstrated to induce hematological remission in CNL. Hasle et al. provided an account of a young patient in blast phase CNL having attained a second chronic phase following AML-type chemotherapy induction [4]. However, the preponderance of the literature suggests that most patients will either be refractory to this approach or eventually succumb to treatment-related mortality [9, 29, 50]. Induction chemotherapy is thus not recommended as standard therapy in CNL.

Interventional procedures

Splenic irradiation and splenectomy

Both splenic irradiation and splenectomy have been used since the 1970s for the management of symptomatic splenomegaly in CNL [31, 51]. However, anecdotal reports of worsening neutrophilia post-splenectomy have rendered this modality obsolete. Splenic irradiation may still be considered a possible modality to address symptomatic splenomegaly in patients failing conventional therapies [4, 51].

Hematopoietic stem cell transplant

Though the previously detailed pharmacologic agents may provide temporary disease stability or in rare instances durable remissions as may be the case with IFN, none has proven disease-modifying effects. Unfortunately, literature on HSCT in CNL remains scarce. The first report of transplant in CNL was in 1996 by Hasle et al. describing long-term remissions in 2 young patients (15 and 25 years old) with CNL having undergone allogeneic hematopoietic stem cell transplant [4]. Both were in remission 6.5 and 4.5 years post-HSCT, respectively. Most cases of HSCT in CNL thus far have consisted of sibling donors, though limited reports relay cases of unrelated donors [11, 52, 53]. Transplant has been described in all phases of the disease with those transplanted in blast phase, as expected, having worse outcomes [29, 52]. In a review of transplant outcomes in CNL, 71% of patients transplanted in chronic phase had durable remissions lasting beyond 7 months compared to shorter remission periods for those transplanted in accelerated phase [54].

A contemporary retrospective nationwide study of allo-HSCT in Japan assessed data from 14 atypical chronic myeloid leukemia (aCML) and 5 CNL patients transplanted between 2003 and 2014 [11•]. Most received myeloablative preconditioning regimens and donor sources were primarily alternative donors versus HLA-matched related (n = 14 vs 5, respectively). One-year overall survival rates were 40% which, given the often-dismal prognosis with alternate therapies, substantiates the salutary effect of allo-HSCT on survival in CNL. Further supporting these findings are recent population-based data from Ruan et al. [3•]. This large-scale retrospective study disclosed frontline allo-HSCT in CNL patients as effective in prolonging long-term survival as both patients managed with this approach remained alive after 5 years, though both were young (< 65 years old) with no major comorbidities (both had Charlson-Deyo scores of 0) [3].

CSF3R mutations may serve as a biomarker for disease relapse post-transplant when present at baseline. It may, accordingly, be reasonable to monitor *CSF3R* mutant allele burden at regular intervals following HSCT [55].

Given the restricted efficacy of currently available therapeutic agents and the often rapidly fatal course observed in CNL, we recommend that eligible patients be considered for HSCT, particularly if they display high-risk features as defined by our institution's prognostic model [10•]. As the concept of "risk-adapted therapy" does not formally apply to CNL, the general directive of "transplant if you can" is likely regarded as the safest approach in managing this aggressive disease, at least until bona fide disease-modifying drugs become available. The optimal timing of transplant remains an unanswered question, though some data preferentially support transplanting in the chronic phase of the disease [29, 52, 54]. It may therefore be advisable to evaluate and deploy donor searches earlier versus later in the disease course, prior to disease progression. Scarce data also exist on HSCT outcomes using alternative stem cell sources and non-myeloablative regimens, and further studies are required to address the safety and feasibility of these approaches.

Emerging therapies

Novel therapeutic targets

Advances in genomics have translated to a movement towards increasingly molecularly driven targeted therapy in CNL. Uniquely targeting the JAK-STAT pathway is speculated to be ineffective in certain patients, particularly those harboring secondary mutations. The identification of *NRAS* mutations in a portion of CNL patients provided a rationale for MEK inhibitor therapy with drugs such as trametinib [45, 56, 57]. In a study by Rohrabaugh et al., MAP kinase signaling was determined to be crucial to leukemogenesis in a mouse model of CNL and importantly, inhibition of MEK1/2 by trametinib alone was found to be sufficient to suppress leukemia in this model [45]. Furthermore, trametinib is expected to be equally effective in *CSF3R* proximal and compound mutants [58]. Overall, these data establish MAPK signaling pathway inhibition as a novel and promising target in CNL and provide a rationale for future studies. While no clinical trials with this or other investigational agents are currently ongoing to our knowledge, the availability of trametinib for other indications and

its potential to bypass JAK inhibitor resistance make this an interesting potential therapeutic consideration for CNL.

Summary

Chronic neutrophilic leukemia is a rare, often clinically aggressive myeloid malignancy with few effective treatment options. The mainstay of management consists of allogeneic hematopoietic stem cell transplant in eligible patients and pharmacological agents, primarily hydroxyurea, interferon, and, most recently, JAK inhibitors, as alternatives to either transplant or pre-transplant to control myeloproliferation and associated symptoms. While oncogenic driver mutations in *CSF3R* remain the genetic signature of CNL, rapidly emerging genomic and transcriptomic profiling data have redefined disease pathogenesis and clonal evolution, and provided potential molecular mechanisms for resistance to therapy. Significant challenges in CNL management remain, including determining optimal candidates for and timing of HSCT, clarifying the role of pharmacologic agents peri-transplant, refining and optimizing prognostic risk and drug resistance modeling, and expanding drug targets with emphasis on formulations that eradicate mutant cells. As detailed genetic features of CNL are exposed and more innovative and comprehensive studies are conducted, there is mounting potential for meaningful therapeutic progress in CNL that will likely increasingly rely on genetically driven, personalized therapeutic strategies.

Compliance with Ethical Standards

Conflict of Interest

Natasha Szubera declares that she has no conflict of interest. Ayalew Tefferic declares that she has no conflict of interest.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

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- •• Of major importance

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