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Precision immunotherapy, mutational landscape, and emerging tools to optimize clinical outcomes in patients with classical myeloproliferative neoplasms

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Abstract

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AUTHORSHIP CONTRIBUTIONS

TIM, RK, TL, RM, RS, MM, OA, GS, and RV designed the outline strategy of the manuscript, analyzed and interpreted the data, and wrote the first draft of the manuscript. All authors participated in writing significant sections of the paper, and all approve the final version of the manuscript.

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Following the 47th American Society of Hematology Meeting in 2005, the late John Goldman and Tariq Mughal commenced a conference, the 1st Post-ASH Workshop, which brought together clinicians and scientists, to accelerate the adoption of new therapies for patients with myeloproliferative neoplasms (MPNs). The concept began with recognition of the CML success story following imatinib therapy, the discovery of *JAK2*^{V617F}, and the demonstration that BCR-ABL1-negative MPNs are driven by abnormal JAK2 activation. This review is based on the presentations and deliberations at the XIIth Post-ASH Workshop on *BCR-ABL1* positive and negative MPNs that took place on December 12 to 13, 2017, in Atlanta, Georgia, immediately following the 59th American Society of Hematology Meeting. We have selected some of the translational research and clinical topics, rather than an account of the proceedings. We discuss the role of immunotherapy in MPNs and the impact of the mutational landscape on TKI treatment in CML. We also consider how we might reduce TKI cardiovascular side effects, the potential role of nutrition as adjunctive nonpharmacologic intervention to reduce chronic inflammation in MPNs, and novel investigational therapies for MPNs.

Keywords

Chronic myeloid neoplasms; immunotherapy; genomics; treatment

1 | INTRODUCTION

Following the remarkable success of the study and *BCR-ABL1* tyrosine kinase inhibitor (TKI) therapy of CML, we have gained an enhanced understanding of the molecular biology of the other classical myeloproliferative neoplasms (MPNs), some of which has now translated into survival benefits for MPN patients. This review is based on the presentations and deliberations at the 12th Post-ASH Workshop on *BCR-ABL1* positive and negative MPNs that took place on December 12 to 13, 2017, in Atlanta, Georgia, immediately following the 59th American Society of Hematology Annual Meeting. Rather than presenting a resume of the workshop proceedings, we discuss some of the translational research and clinical topics in greater detail. We discuss the role of immunotherapy in MPNs and the impact of the mutational landscape on TKI treatment in CML. We also consider how we might reduce TKI cardiovascular side effects, the potential role of nutrition as adjunctive nonpharmacologic intervention to reduce chronic inflammation in MPNs, and novel investigational therapies for MPNs.

2 | TOWARDS PRECISION IMMUNOTHERAPY FOR MPNs

The landscapes of somatic mutations of many cancer types have been defined by next-generation sequencing (NGS) methods. What has become clear is that tumors display genetic heterogeneity not only within a diagnostic class but also within the tumor itself.^{1,2} Recurrent mutations serve diagnostic, prognostic, and biomarker purposes, but many other mutations are unique to each tumor. Treatment strategies are needed that can target cancer cells with their genetic heterogeneity. The immune system is designed to eliminate cells carrying “non-self” features, and recent success of immunotherapies of cancer demonstrated the feasibility of this approach.³ Because targeted immunotherapy relies on defined tumor

antigens against which immune response is triggered, identification of global antigenic features of tumors is necessary.^{4,5} Myeloproliferative neoplasms are characterized by chronic overproduction of terminally differentiated blood cells, predisposition to thrombosis and acute leukemia, and 3 driver mutations in *JAK2*, *CALR*, and *MPL* genes, which cause the disease (Figure 1).^{6,7} Many other MPN-associated mutations have been identified that play different roles in the pathogenesis. The mutational landscape of MPN offers a number of targets for monoclonal antibody development and engineered immune cells that can selectively target the antigen-expressing cancer cells and leave the normal hematopoietic cells unharmed.⁸ Efforts are also assessing the concept of individualized mutanome vaccines and RNA-based poly-neo-epitope approaches to mobilize immunity against cancer mutations.⁹

The frameshift mutations of *CALR* in MPN that generate a unique C-terminal amino acid sequence are the most recurrent neoantigens in MPN.^{10,11} A number of studies have shown that the mutant *CALR* activates the thrombopoietin receptor *MPL* and that the active *CALR/MPL* complex traffics to the cell surface. The novel (approximately 40 amino acid long) C-terminus of the mutant *CALR* as well as its surface expression on myeloid cells makes it an ideal target for immunotherapy.^{12,13} The mutated forms of *CALR* are immunogenic in mice and rabbits, and monoclonal antibodies could be generated against C-terminal amino acids derived from the mutant *CALR* using hybridoma technology (mice) and phage display (rabbits) (Figure 2).¹⁴ These antibodies recognize specifically the mutant *CALR* and detect the expression of the mutant *CALR* protein on the cell surface. The anti-mutant-*CALR* antibodies can be used for diagnostic purposes because all of the so far identified *CALR* mutants (over 50 different indels) have an identical C-terminus. Using an ELISA assay, one could identify *CALR* mutant-positive patients from healthy and *CALR*-negative cases. The detection of variable insertion/deletion *CALR* mutants at DNA level requires 7 qPCR assays to cover all possible mutants, or PCR product sizing and sequencing is required for DNA-based tests. On protein level, a single immunoassay is sufficient using serum or plasma. In *CALR*-positive MPNs, the mutant *CALR* is secreted at variable levels by the cancer cells. Quantification of secreted mutant *CALR* will be an important parameter for considering “sink effect” during antibody therapy.

Anti-*CALR* antibodies or their fragments can be configured into various formats including naked antibodies (inducing antitumor effect via complement or NK cell activation), drug-conjugated antibodies, chimer antigen receptors, and bispecific antibodies. The feasibility of antibody therapy of *CALR*-positive MPNs is currently being tested in vivo in a conditional knock-in mouse model expressing the human *CALR* exon 9 with the del52 mutation.

Chronic MPN often develops into an accelerated disease and acute leukemia. The somatic mutation number increases, and patients develop a complex clonal hierarchy with large number of somatic mutations. Each patient at the leukemic phase is genetically unique, and only personalized immunotherapy can target the tumor cell at this stage. Using transcriptome sequencing, both chronic phase and leukemic phase patients were studied for the presence of mutations and splicing aberrations. Many of these variants were predicted to produce peptides with strong affinity to common HLA alleles. These candidate neoantigens are being evaluated for immunogenicity in healthy donors and MPN patients.

3 | SCREENING AND MONITORING OF *BCR-ABL1* KINASE DOMAIN MUTATIONS FOR APPROPRIATE SELECTION AND DOSE OF TKIs

The role of NGS for mutation screening in *BCR-ABL1*-positive leukemias is well established.^{15–17} Diagnostic approaches relying on different NGS platforms permit the assessment of low-level mutations in the *ABL1* kinase domain (KD) approximately 1 log below the detection limit of Sanger sequencing, the previous gold standard of mutation analysis.¹⁸ Although different publications provided evidence for the clinical impact of low-level mutations,^{19–21} quantitative monitoring of mutant subclone expansion during TKI treatment may provide more reliable information for the impending onset of resistant disease.²² Guidelines provided by the NCCN and the ELN include specific recommendations for the use of individual TKIs in the presence of some of the most commonly occurring *ABL1* KD mutations. In the presence of other mutations in the kinase domain, clinicians often rely on published “heat maps” indicating the expected responsiveness of various mutations to the available TKIs. The existing heat maps were established on the basis of in vitro analysis of a cell line, most commonly the murine Ba/F3 cells, containing individual mutant *BCR-ABL1* constructs. Translation of the TKI responsiveness indicated by individual heat maps into the clinical setting must be performed with great caution because the differences between published data are considerable. Indeed, careful analysis of the literature can reveal data on the responsiveness of individual mutations to specific TKIs ranging from highly sensitive to highly resistant, rendering the interpretation and translation into the clinical setting difficult. Recent observations indicate that some published data overestimate the in vitro resistance of individual mutations to specific TKIs, and this phenomenon might be attributable to a technical problem related to the generation of mutant *BCR-ABL1* cell lines. Lentiviral or transposon-based transfer of mutant *BCR-ABL1* constructs into a cell line can result in multiple insertions into the genome which leads to artificially elevated IC50 values for individual TKIs when such cells are used in in vitro sensitivity assays.²³ The bias in the readout can be avoided by specific selection of cells containing insertion of the mutant *BCR-ABL1* constructs at a single site prior to testing of TKI responsiveness in vitro. Awareness of this problem is important for the generation of data amenable to translation into the clinical setting.

Some patients with *BCR-ABL1*-positive leukemia display 2 or more mutations in the *BCR-ABL1* KD which can be present in different cells (polyclonal constellation) or in the same cell (compound constellation). Compound mutations (CMs) were demonstrated to display high resistance to all available TKIs, including the third-generation compound ponatinib. Recent data suggest, however, that only certain compound mutations provide insurmountable resistance to this TKI. These include particularly CMs including the gatekeeper mutation T315I or the adjacent mutation F317L.^{24,25} Such mutations would require concentrations of ponatinib that are not achievable in the clinical setting, even when using the highest possible dosage of 45 mg/day. By contrast, a number of other compound mutations may be controlled by any dose of ponatinib down to 15 mg/day. Some compound mutations, however, display intermediate levels of resistance that can only be controlled by higher doses of ponatinib.²⁵ This consideration is important because the serious side effects associated with ponatinib treatment appear to be dose-related, thus leading to the general

tendency of limiting the daily dose of this compound. Hence, recent data suggest that the clinical use of mutation testing by NGS goes beyond the selection of an appropriate TKI and response monitoring of individual mutant subclones to TKI treatment in vivo. In some instances, identification of specific mutations may also guide appropriate dosing of the selected TKI.²⁵

4 | ASSESSMENT OF CARDIOVASCULAR RISK FACTORS FOR PATIENTS WITH CML IN CHRONIC PHASE RECEIVING TKIs

The past few years have witnessed an important paradigm shift in the treatment of patients with CML in chronic phase, with a greater emphasis on reducing the risk of experiencing serious and potentially life-threatening side effects, such as cardiovascular events, and discontinuing TKIs safely and effectively. The remarkable success of several *BCR-ABL1* TKIs, often for long periods of time, results in most patients having a near-normal or normal life expectancy.^{26–28} Second-generation and third-generation TKIs have greater potency resulting in earlier and deeper molecular responses, but are also associated with important serious adverse events, in particular cardiovascular and pulmonary, compared with imatinib treatment.^{29–34} At present, there is, however, no significant difference in overall survival with the more potent TKIs, including those who achieve sustained deep molecular responses, underscoring the need to balance treatment-related risks against better CML-related responses. About 40% of patients who have been in sustained long-term complete molecular remission are able to discontinue TKI therapy, and therefore minimize long-term TKI toxicity, both physical and financial.^{35,36} It is possible, but not certain, that this treatment-free remission may be more likely with the next-generation TKIs, but this will require further follow-up. The significant impact on overall survival, but not CML responses, of comorbidities, has been observed in studies such as the German CML IV study, which used the Charlson comorbidity index.³⁷

Randomized prospective studies have observed the occurrence of TKI-related cardiovascular serious events in CML patients with pre-existing cardiac conditions or risk factors, including adverse metabolic changes, diabetes mellitus, and lipid profile changes.^{38–40} As an illustration, the ENESTnd study demonstrated a third of the study cohort to have intermediate or high-risk Framingham risk score, and comprised 70% of those who experienced nilotinib-related cardiac events; the EPIC study noted that 10 of 11 CML patients who developed ponatinib-related arterial events had 1 or more cardiac risk factors, or a history of cardiovascular disease.^{30,41} Meta-analyses and population-based studies clarify such risks as class effects or specific to certain TKIs.^{42–44} In the regard, it is reassuring to note that following 11 years of follow-up, both the safety and efficacy of imatinib, was confirmed recently.⁴⁵ Serious adverse events were uncommon and occurred largely in the first 12 months of imatinib therapy.

Patients commencing or switching to nilotinib and ponatinib have the most robust recommendations for baseline and subsequent interval testing of indicators of vascular disease, such as ankle-brachial index measurement, and metabolic studies; echocardiography may be of the greatest benefit in dasatinib-treated patients as pulmonary artery pressure can

often be estimated as well as the absence of preexistent pericardial and pleural effusions. Clearly in efforts to effectively manage comorbidities and minimize treatment-related adverse events, additional tools, such as the Framingham risk model and the European Society of Cardiology score, and novel treatment approaches to suppress multiresistant CML subclones, such as “TKI rotation therapy,” are being tested.^{46,47} A multidisciplinary team approach, including cardiology specialists conversant with the TKI-associated vascular complications, is also desirable. Finally, it is likely that molecular risk factors, including the presence of age-related somatic mutations, have a role in vascular side effects.⁴⁸

5 | THE POTENTIAL ROLE OF NUTRITION AS ADJUNCTIVE NONPHARMACOLOGIC INTERVENTION TO REDUCE INFLAMMATION IN THE MPNs

Patients with MPNs have abnormal cytokine expression that contributes to symptom burden, nutritional deficiencies, and disease progression.^{49–53} Therapeutic interventions are limited among patients with indolent disease and focused on reducing thrombotic risk. To date, no studies have evaluated the nutritional needs or preferences of MPN patients regarding dietary change. Scherber and colleagues assessed the needs and preferences of nutrition and supplement use in MPN patients using an internet-based survey, hosted by the Mayo Clinic Survey Research Center and promoted on multiple MPN-based forums, social media (Figure 3), and a focus group meeting in 2017 in Irvine, CA. Survey included data on demographics, MPN characteristics, nutritional habits, supplement use, and symptom burden using the MPN-SAF TSS/MPN-10.⁵⁴ The study cohort comprised of 1329 MPN patients in 37 countries. Some of the highlights of the survey were notable for a high prevalence of preexisting dietary change and immense interest in a dietary intervention. Among respondents, 34.0% of patients endorsed already using diet to help control their symptoms or MPN disease. Patients used a variety of resources for nutritional education including books (27.7%), websites (26.1%), health care providers (22.3%), online forums (21.7%), friends (12.1%), nutritionists (9.7%), phone or tablet applications (8.2%), and videos (3.7%). Almost all patients (96.2%) were willing to restrict their diet if it helped to control symptom burden and or restrict their diet if it could stabilize or help prevent progression their MPN (98%). When analyzing as a dichotomous variable (at least once per week intake versus no intake), at least once per week fast food, fried foods, and soda associated with significantly higher symptom burden ($P < .05$). When evaluating as a continuous variable, fast food, premade snacks, soda, refined sugar, and tacos all associated with worsened symptom score ($P < .05$).

Seven themes were identified during the focus group, including (i) patients' MPN disease and symptoms impact their dietary choices, (ii) patients are concerned about the lack of resources regarding diet, (iii) MPN patients experience common barriers to dietary change (ie, lack of time, difficulties with food choices), (iv) motivators for dietary change are common and usually disease-related, (v) MPN patients prefer a tailored dietary intervention, (vi) supplement use is common in addition to dietary intervention, and (vii) MPN patients are enthusiastic and optimistic about nonpharmacologic interventions.

The findings of these investigations suggest a promising role of nutritional adjunctive therapy in MPNs, through the immense interest by the MPN patient community and the associations between dietary intake of foods considered pro-inflammatory and symptom burden. Currently, no consensus recommendations or standard of care exists for the nutritional management of MPN patients. The benefits of a nutritional intervention that emphasizes the intake of foods rich in anti-inflammatory properties would be suspected to be twofold: (1) improvement in symptom burden and (2) reduction in inflammation. Previous dietary interventions studied primarily within a cardiovascular risk reduction setting have found reductions in inflammatory markers (eg, TNF- α , IL-6, and CRP) and thrombotic markers (eg, homo-cysteine, fibrinogen). It is notable that these same markers appear to play a role in MPN disease. IL-6 has been found to be increased in MPN patients,⁵⁵ TNF- α has been found to have a role in bone marrow fibrosis as well as selective advantage of *JAK2*^{V617F} allele burden,^{53,56} and high sensitivity CRP can be elevated in MPN patients and is associated with thrombotic risk.⁵⁷ These reductions in thrombotic markers observed with previous dietary interventions are of particular relevance to the treatment of indolent MPNs, where the primary risk of death or disability is because of thrombotic events.⁵⁸ A prospective feasibility study of dietary change in MPN patients with an emphasis on foods rich in anti-inflammatory properties is planned. There is also interest in assessing other lifestyle features associated with MPNs, such as the recently observed positive associations between the level of physical activity and QoL, independently of fatigue being present.⁵⁹

6 | INVESTIGATIONAL THERAPIES FOR CLASSICAL MPNs in 2018

Clearly, following the impressive success of *BCR-ABL1* TKIs for patients with CML, there was considerable optimism as the *JAK2* inhibitors entered clinical trials for MPNs. But a decade later, the results, for the most part, suggest a qualified success with significant symptomatic benefit for patients with myelofibrosis (MF) and polycythemia vera (PV), but neither major change in the natural history nor a significant impact on the *JAK2*^{V617F} allelic burden. Ruxolitinib, a *JAK1* and a type II *JAK2* inhibitor with a short half-life, remains the only approved therapy for patients with intermediate and high-risk MF, and for patients with PV refractory or intolerant to hydroxyurea (hydroxycarbamide). The drug is a potent anti-inflammatory agent also and improves the clinical state and survival of patients with MF. The majority of other type I *JAK2* inhibitors have had their clinical development discontinued largely because of the emergence of serious neurotoxicity. Currently, pacritinib remains in phase 3 studies and fedratinib is now being reevaluated.⁶⁰

Fedratinib was previously shown to be superior to placebo for control of splenomegaly and symptoms in patients with MF in the Jakarta I study.⁶¹ Additionally, fedratinib had been shown to be active in the second-line setting for individuals who had previously been on ruxolitinib in the Jakarta II study.⁶² Further development of fedratinib was discontinued in November 2013 because of concerns of *Wernicke's* encephalopathy (WE) observed in 8 of the 877 total patients treated with fedratinib. However, a reanalysis in late 2017 suggested that only 1 of these 8 patients met the diagnostic criteria for WE. Furthermore, even this solitary patient might well have had WE prior to study entry, which worsened on fedratinib and resolved with treatment with IV thiamine.⁶³ Among the other cases, they ranged from

unconfirmed to clearly not having WE, and on the basis of these data and further review, the FDA lifted the clinical hold for fedratinib, which is now being redeveloped.

Pacritinib, previously shown to be active in the PERSIST-1 and PERSIST-2 studies, is undergoing further development with refinement of optimal dosage.^{64,65} Indeed, the recently published phase 3 study results, in which pacritinib 200 mg twice daily was found to be significantly better than best available therapy, including ruxolitinib, for reducing splenomegaly and clinical symptoms in patients with MF and thrombocytopenia, for both previously untreated and those who had received prior ruxolitinib. Momelotinib was reported in results of the SIMPLIFY-1 study in late 2017 to be not inferior to ruxolitinib for reduction of splenomegaly, slightly inferior for control of MF symptom burden, and active for anemia as frontline therapy for patients with MF.⁶⁶ This latter trial not having met its primary endpoint leaves an uncertain future for momelotinib.⁶⁷

The use of long-acting interferons continues to be an area of great interest for patients with MPNs with 2 large studies discussed at the 2017 ASH and Post-ASH meetings. The first was the 2-year follow-up data of ropegylated interferon alpha 2b versus hydroxyurea as frontline therapy for patients with PV.⁶⁸ This study demonstrated that the pegylated interferon was likely superior to hydroxyurea for achievement of complete hematologic response after 2 years and likely improved molecular responses (both were equivalent through the first 12 months of the trial). The second study was a second-line study of pegylated interferon alpha 2a in patients with PV or essential thrombocythemia who had previously failed hydroxyurea.⁶⁹ This study conducted by the MPN Research Consortium demonstrated over 60% response rate as second-line therapy for these patients. In aggregate, the studies continued to demonstrate the safety and efficacy of pegylated interferons for therapy of patients with particularly PV and may well lead to commercial availability in the near future.

In addition to JAK inhibition and interferons, there are multiple additional pathways currently being investigated using a range of approaches in the second-line setting for both MF and PV (Table 1).^{70–78} These agents range in goals from improving the anemia of patients with MF, either alone or in combination with ruxolitinib, to inhibition of the hedgehog, aurora kinase, SMAC, HDAC, and MDM2 pathways. The next generation of JAK2-specific drugs includes allosteric inhibitors, such as LS104 and ON044580, which have a greater specificity for *JAK2*^{V617F} and are inhibitory in a non-ATP-competitive manner.

7 | CONCLUSION

Arguably, CML is the 1 real success in targeted therapy, contingent upon *BCR-ABL1* being the founder lesion in every cell, and unlike most other cancers, including other subtypes of MPNs, has minimal genetic diversity. Resistant and compound mutants have been an issue, but many patients are now able to achieve second and subsequent remissions, following a switch to an alternative TKI or an allogeneic stem cell transplant. To maintain such success, we need firm strategies in clinics to counter potential TKI-related toxicities and to assess risk factors carefully. In comparison, clinical progress for other subtypes of MPNs has been

more limited, but there have been therapy advances for MF and PV, with several type I JAK2 and next-generation JAK inhibitors in clinical trials.

In contrast to CML, the BCR-ABL1-negative MPNs do demonstrate significant genetic diversity, with the somatic mutation number increasing alongside a complex clonal hierarchy, as the disease progresses from a chronic phase to acute leukemia. Many of these variants are thought to produce neoantigens which could be precision immunotherapy targets. Immune responses against CALR mutants, in particular CALR exon9, and spontaneous T cell responses against PD-L1 in MPNs have also been observed, and these are also potential targets for immunotherapy.⁷⁹ It is also timely to assess the role of nutrition as a nonpharmacologic intervention to reduce chronic inflammation in MPNs.

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FIGURE 1.
Driver mutations in BCR-ABL1-negative myeloproliferative neoplasms

FIGURE 2.

Surface expression of the mutant CALR-thrombopoietin receptor (MPL) complex in UT-7/Tpo cells engineered with CRISPR/Cas9 mutagenesis to carry a CALR frameshift mutation. Immunofluorescence imaging using proximity ligation assay performed with anti-MPL and anti-mutant-CALR antibodies

FIGURE 3.
Countries involved in the NUTRIENT Survey in February 2017

TABLE 1

Novel pathways targeted in myeloproliferative neoplasm trials

Drug/Pathway	Disease/Setting	Reference
Sotatercept/ACTRIIa	MF anemia	Bose et al ⁷⁰
Glasdegib/Hedgehog	MF second line	Gerds et al ⁷¹
Alisertib/Aurora Kinase	MF second line	Gangat et al ⁷²
SL-401/rIL3+ dipTox	MF/CMML second line	Patnaik et al ⁷³
LCL 161/SMAC	MF second line	Pemmaraju et al ⁷⁴
Pracinostat/HDAC	MF second line	Bose et al ⁷⁵
Vismodegib + RUX/Hedgehog	MF second line	Couban et al ⁷⁶
Givinostat/HDAC	PV second line	Rambaldi et al ⁷⁷
Idasanutlin/MDM2	PV second line	Mascarenhas et al ⁷⁸