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

Journal
Hematological Oncology, 36(5)

ISSN
0278-0232

Authors
Mughal, Tariq I
Lion, Thomas
Abdel-Wahab, Omar
et al.

Publication Date
2018-12-01

DOI
10.1002/hon.2537

Peer reviewed

HHS Public Access

Author manuscript

Hematol Oncol. Author manuscript; available in PMC 2019 July 08.

Published in final edited form as:

Hematol Oncol. 2018 December ; 36(5): 740–748. doi:10.1002/hon.2537.

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

Tariq I. Mughal[1], **Thomas Lion**[2], **Omar Abdel-Wahab**[3], **Ruben Mesa**[4], **Robyn M. Scherber**[5], **Danilo Perrotti**[6], **Michael Mauro**[3], **Srdan Verstovsek**[7], **Giuseppe Saglio**[8], **Richard A. Van Etten**[9], and **Robert Kralovics**[10,11]

[1]Tufts University Cancer Center, Boston, MA, USA

[2]Children's Cancer Research Institute, Medical University of Vienna, Vienna, Austria

[3]Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]UT Health San Antonio Cancer Center, San Antonio, TX, USA

[5]Oregan Health and Science University, Portland, OR, USA

[6]Greenebaum Cancer Center, University of Maryland, Baltimore, MD, USA

[7]MD Anderson Cancer Center, Houston, TX, USA

[8]Orbassano University Hospital, Turin, Italy

[9]University ofCalifornia Irvine, Irvine, CA, USA

[10]Research Center for Molecular Medicine of the Austrian Academy of Science, Vienna, Austria

[11]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

Abstract

Correspondence: Tariq I. Mughal, Division of Hematology and Oncology, Tufts University Cancer Center, Boston, MA 02111, USA. tariq.mughal@tufts.edu.

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.

CONFLICT OF INTEREST

O. Abdel-Wahab: No relevant disclosures
R. Kralovics: Consultancy: AOP Orphan, PharmaEssentia, MyeloPro Diagnostics and Research
T. Lion: No relevant disclosures
M. Mauro: Consultancy: Novartis, Bristol-Myers Squibb, Pfizer, Takeda
R. Mesa: Research funding: Incyte, Lilly, NS Pharma, Sanofi, Gilead, CTI, Genentech
T.I. Mughal: Scientific Advisory Boards: Sanofi
D. Perrotti: No relevant disclosures
G. Saglio: Consultancy: Bristol-Myers Squibb; Research funding: Novartis
R. Scherber: No relevant disclosures
R.A. Van Etten: Scientific Advisory Boards: Bristol Myers-Squibb, Pfizer
S. Verstovsek: Research funding: Incyte Corporation, Roche, Astra Zeneca, Lilly Oncology, NS Pharma, Bristol Myers Squibb, Celgene, Gilead, Seattle Genetics, Promedior, CTI BioPharma Corp., Galena BioPharma, Pfizer, Genentech, Blueprint Medicines Corp.

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 myeloroliferative 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 Framing-ham 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 IJAK2 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 revaluated.[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 alternativeTKI 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-ABLl-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.

ACKNOWLEDGEMENTS

The XIIth Post-ASH CML-MPN workshop was composed of O Abdel-Wahab, T Barbui, P Bose, C Jamieson, J Crispino, M Deininger, A Eiring, S Elf, A Fleishmann, R Gale, O Hantschel, R Hehlmann, S Koschmieder, R Kralovics, T Lion, G Martinelli, R Mesa, T Mughal, O Ottman, D Perrotti, T Odenike, H Gisslinger, JJ Kiladjian, M Mauro, J Maxson, A Mead, R Rampal, G Saglio, Y Rok-Do, S Sausselle, R Scherber, T Shimoda, R Skoda, R Silver, T Skorski, E Solary, S Soverini, R Van Etten and S Verstovsek. The members wish to thank Alpa Parmar for her insights and organizational skills and Incyte Corporation, Novartis Oncology, Roche Pharma and Alpine Oncology Foundation for their unrestricted grant support.

Funding information

Alpine Oncology Foundation; Roche Pharma; Novartis Oncology; Incyte Corporation

REFERENCES

1. McGranahan N, Swanton C. Biological and therapeutical impact of intratumor heterogeneity in cancer evolution. Cancer Cell. 2015;27(1):15–26. [PubMed: 25584892]
2. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;11:214–218.
3. Olivera F Cancer immunology. N Engl J Med. 2008;358:2704–2715. [PubMed: 18565863]
4. Coulie PG, Van der Eynde BJ, Van der Bruggen P, BoonT. Tumor antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer. 2014;14(2):135–146. [PubMed: 24457417]
5. Schumacher TN, Schreider RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230): 69–74. [PubMed: 25838375]
6. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproloferative neoplasms. N Engl J Med. 2005;352(17):1779–1790. [PubMed: 15858187]
7. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017;129(6):667–679. [PubMed: 28028029]
8. Schischlik F, Kralovics R. Mutations in myeloprloferative neoplasms - their significance and clinical use. Expert Rev Hematol. 2017;10(11):961–973. [PubMed: 28914569]
9. Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222–226. 10.1038/nature23003 [PubMed: 28678784]
10. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–1390. [PubMed: 24325356]
11. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25):2391–2405. [PubMed: 24325359]

12. Holmström MO, Riley CH, Svane IM, Hasselbalch HC, Anderson MH. The CALR-exon 9 mutations are shared neoantigens in patients with CALR mutants chronic myeloproliferative neopalsms. Leykemia. 2016;30(12):2413–2416.
13. Holmström MO, Martinenaite E, Ahmad DM, et al. The Calreticulin (CALR) exon 9 mutations are promising targets for cancer immunotherapy. Leukemia. 2018;32(2):429–437. [PubMed: 28676668]
14. Kralovics R Anti-mutant calreticulin antbodies and their use in the diagnosis and therapy of myeloid malignancies. US Patent App. 2017 15/532,453
15. Soverini S, de Benedittis C, Machova Polakova K, et al. Unraveling the complexity of tyrosine kinase inhibitor-resistant populations by ultra-deep sequencing of the BCR-ABL kinase domain. Blood. 1634–1648;2013:1122.
16. Kastner R, Zopf A, Preuner S, et al. Rapid identification of compound mutations in patients with Philadelphia-positive leukaemias by long-range next generation sequencing. Eur J Cancer. 2014;50(4):793–800. [PubMed: 24365090]
17. Preuner S, Barna A, Frommlet, et al. Quantitative Analysis of Mutant Subclones in Chronic Myeloid Leukemia: Comparison of Different Methodological Approaches. Int J Mol Sci. 2016;17(5):642 10.3390/ijms17050642
18. Baccarani M, Castagnetti F, Gugliotta G, et al. A review of the European LeukemiaNet recommendations for the management of CML. Ann Hematol. 2015;94(Suppl. 2):S141–S147. [PubMed: 25814080]
19. Parker WT, Ho M, Scott HS, Hughes TP, Branford S. Poor response to second-line kinase inhibitors in chronic myeloid leukemia patients with multiple low-level mutations, irrespective of their resistance profile. Blood. 2012;119(10):2234–2238. [PubMed: 22210874]
20. Kizilors A, Elena Crisa E, Lea N, et al. Impact of finding of low level kinase domain mutations using ultra deep next generation sequencing in patients with chronic phase CML. Blood. 2015;126:347.
21. Parker WT, Yeung DTO, Yeoman AL, et al. The impact of multiple low-level BCR-ABL1 mutations on response to ponatinib. Blood. 2016;127(15):1870–1880. [PubMed: 26773037]
22. Preuner S, Mitterbauer G, Mannhalter C, et al. Quantitative monitoring of BCR/ABL1 mutants for surveillance of subclone-evolution, - expansion, and -depletion in chronic myeloid leukaemia. Eur J Cancer. 2012;48(2):233–236. [PubMed: 21955823]
23. Byrgazov K, Lucini CB, Berkowitsch B, et al. Transposon-mediated generation of BCR-ABL1-expressing transgenic cell lines for unbiased sensitivity testing of tyrosine kinase inhibitors. Oncotarget. 2016;7(47):78083–78094. [PubMed: 27801667]
24. Zabriskie MS, Eide CA, Tantravahi SK, et al. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Philadelphia chromosome-positive. Cancer Cell. 2014;26(3):428–442. [PubMed: 25132497]
25. Byrgazov K, Lucini CB, Valent P, Hantschel O, Lion T. BCR-ABL1 compound mutants display differential and dose-dependent responses to ponatinib. Haematologica. 2018;103(1):e10–e12. [PubMed: 28983061]
26. Pfirrmann M, Baccarani M, Saussele S, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56. [PubMed: 26416462]
27. Mughal TI, Dieninger M, Radich JR, et al. Chronic myeloid leukemia: Reminscences and dreams. Haematologica. 2016;101(5):541–558. [PubMed: 27132280]
28. Hughes TP, Ross DM. Moving treatment-free remission into mainstream clinical practice in CML. Blood. 2016;128(1):17–23. [PubMed: 27013442]
29. Hochhaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30(5):1044–1054. [PubMed: 26837842]
30. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naive chronic myeloid leukemia patients trial. J Clin Oncol. 2016;34(20):2333–2340. [PubMed: 27217448]

31. Lipton JH, Chuah C, Guerci-Bresler A, et al. Ponatinib versus imatinib for newly diagnosed chronic myeloid leukaemia: an international, randomised, open label, phase 3 trial. Lancet Oncol. 2016;17(5):612–621. [PubMed: 27083332]
32. Cortes JE, Gambacorti-Passerini C, Deininger MW, et al. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–237. [PubMed: 29091516]
33. Cortes JE, Jean Khoury H, Kantarjian H, et al. Long-term evaluation of cardiac and vascular toxicity in patients with Philadelphia chromo-some-positive leukemias treated with bosutinib. Am J Hematol. 2016;91(6):606–616. [PubMed: 26971533]
34. Talpaz M, Saglio G, Atallah E, Rousselot P. Dasatinib dose management for treatment of chronic myeloid leukemia. Cancer. 2018 1 25;doi: 10.1002/cncr.31232
35. Mughal TI, Gotlib J, Mesa R, et al. Recent advances in the genomics and therapy of *BCR-ABL1* -positive and -negative chronic myeloproliferative neoplasms. Leuk Res. 2018;67:67–74. [PubMed: 29466766]
36. Mahon FX, Boquiupain C, Kim D-W, et al. Treatment-free remission after second-line nilotinib treatment in patients with chronic myeloid leukemia in chronic phase: results from a single group, phase 2, open-label study. Ann Intern Med. 2018;168(7):461–470. [PubMed: 29459949]
37. Saussele S, Krauss MP, Hehlmann R, et al. Impact of comorbidities on overall survival in patients with chronic myeloid leukemia: results of the randomized CML study IV. Blood. 2015;126(1):42–49. [PubMed: 25918346]
38. Rea D, Mirault T, Cluzeau T, et al. Early onset hypercholesterolemia induced by the 2nd-generation tyrosine kinase inhibitor nilotinib in patients with chronic phase-chronic myeloid leukemia. Haematologica. 2014;99(7):1197–1203. [PubMed: 24658819]
39. Iurlo A, Orsi E, Cattaneo D, et al. Effects of first- and second-generation tyrosine kinase inhibitor therapy on glucose and lipid metabolism in chronic myeloid leukemia patients: a real clinical problem? Oncotarget. 2015;6(32):33944–33951. [PubMed: 26376678]
40. Alahdab F, Wang AT, Elraiyah TA, et al. A systematic review for the screening for peripheral arterial disease in asymptomatic patients. J Vasc Surg. 2015;61(3):42S–53S. [PubMed: 25721066]
41. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369(19):1783–1796. [PubMed: 24180494]
42. Haguet H, Douxfils J, Mullier F, Chatelain C, Graux C, Dogne JM. Risk of arterial and venous occlusive events in chronic myeloid leukemia patients treated with new generation BCR-ABL tyrosine kinase inhibitors: a systematic review and meta-analysis. Expert Opin Drug Saf. 2017;16(1):5–12. [PubMed: 27852118]
43. Chai-Adisaksopha C, Lam W, Hillis C. Major arterial events in patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors: a metaanalysis. Leuk Lymphoma. 2016;57(6): 1300–1310. [PubMed: 26373533]
44. Dahlen T, Edgren G, Lambe M, et al. Cardiovascular events associated with use of tyrosine kinase inhibitors in chronic myeloid leukemia: a population-based cohort study. Ann Intern Med. 2016;165(3):161–166. [PubMed: 27295519]
45. Hochhaus A, Larson RA, Guilhot F, et al. Long-term outcomes of Imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376(10):917–927. [PubMed: 28273028]
46. Valent P, Herndlhofer S, Schneeweiss, et al. TKI rotation-induced persistent deep molecular response in multi-resistant blast crisis of Ph+ CML. Oncotarget. 2017;8(14):23061–23072. [PubMed: 28416739]
47. Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(suppl_4):iv41–iv51. [PubMed: 28881915]
48. Hadzijusufovic E, Hoermann G, Herndlhofer S, et al. Cardiovascular scoring and age-related mutations predict the occurrence of adverse vascular events in CML patients during Nilotinib therapy. Blood. 2017;130:1619.
49. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: the ATTICA study. J Am Coll Cardiol. 2004;44(1):152–158. [PubMed: 15234425]

50. Smidowicz A, Regula J. Effect of nutritional status and dietary patterns on human serum C-reactive protein and interleukin-6 concentrations. Adv Nutr. 2015;6(6):738–747. [PubMed: 26567198]
51. Mesa R, Schwager S, Huang J, et al. Weight loss, splenomegaly, and hypocholesterolemia in myeloproliferative neoplasms: patterns and relevance from the pre JAK2 inhibitor era. Blood. 2009;114:a3918.
52. Mesa R, Schwager S, Huang J, Hussein K, Pardanani A, Tefferi A. Dynamics, and prognostic impact, of weight loss in primary myelofibrosis. Blood. 2008;112:5224.
53. Fleischman AG, Aichberger KJ, Luty SB, et al. TNFalpha facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms. Blood. 2011;118(24):6392–6398. [PubMed: 21860020]
54. Emanuel RM, Dueck AC, Geyer HL, et al. Myeloproliferative neoplasm(MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. J Clin Oncol. 2012;30(33):4098–4103. [PubMed: 23071245]
55. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. J Clin Oncol. 2011;29(10):1356–1363. [PubMed: 21300928]
56. Geyer HL, Dueck AC, Scherber RM, Mesa RA. Impact of inflammation on myeloproliferative neoplasm symptom development. Mediators Inflamm. 2015;2015:284706. [PubMed: 26538823]
57. Barosi G, Massa M, Campanelli R, Fois G, Rosti V. High levels of high sensitivity-C reactive protein (hs-CRP) are associated with older age, chromosomal abnormalities and JAK2V617F mutation with high allele burden in primary myelofibrosis. Blood. 2016;128:A1956.
58. Hultcrantz M, Wilkes SR, Kristinsson SY, et al. Risk and cause of death in patients diagnosed with myeloproliferative neoplasms in Sweden between 1973 and 2005: a population-based study. J Clin Oncol. 2015;33(20):2288–2295. [PubMed: 26033810]
59. Larsen RT, Tang LH, Brochmann N, et al. Associations between fatigue, physical activity, and QoL in patients with myeloproliferative neoplasms. Eur J Haematol. 6 4 2018 10.1111/ejh13048
60. Vainchenker W, Leroy E. L G. JAK inhibitors for the treatment of myeloproliferative neoplasms and other disorders *F1000*. Research. 2018;7:82.
61. Pardanani A, Harrison C, Cortes JE, et al. Safety and efficacy of Fedratinib in patients with primary or secondary myelofibrosis: a randomized clinical trial. JAMA Oncol. 2015;1(5):643–651. [PubMed: 26181658]
62. Harrison CN, Schaap N, Vannucchi AM, et al. Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study. Lancet Haematol. 2017;4(7):e317–e324. [PubMed: 28602585]
63. Harrison C, Mesa M, Jamieson C, Hood, Brewer J. Case Series of Potential Wernicke's Encephalopathy in Patients Treated with Fedratinib. ASHA. 2017;634:A4197.
64. Mesa RA, Vannucchi AM, Mead A, et al. Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): an international, randomised, phase 3 trial. Lancet Haematol. 2017;4(5):e225–e236. [PubMed: 28336242]
65. Mascarenhas J, Hoffman R, Talpaz M, et al. Pacratinib versus bestavailable therapy, including Ruxolotinib, in patients with myelofibrosis. JAMA Oncol. 2018 3 8th/doi: 10.1001/jamaoncol.2017.5818;4(5):652–659. [PubMed: 29522138]
66. Mesa RA, Kiladjian JJ, Catalano JV, et al. SIMPLIFY-1: a phase III randomized trial of Momelotinib versus Ruxolitinib in Janus kinase inhibitor-naive patients with myelofibrosis. J Clin Oncol. 2017;35(34):3844–3850. [PubMed: 28930494]
67. ASH Clinical News. Momelotinib Trial in Myelofibrosis Fails to Meet Primary Endpoint. 2018; https://www.ashclinicalnews.org/news/momelotinib-trial-myelofibrosis-fails-meet-primary-endpoint/. Accessed March 23rd 2018.
68. Gisslinger H, Klade C, Georgiev P, Krochmalczyk D, Gercheva K, Kiladjian J. Ropeginterferon alfa-2b induces high rates of clinical, Hematological and Molecular Responses in Polycythemia

Vera: Two-Year Results from the First Prospective Randomized Controlled Trial. ASH. 2017;634:A320.

69. Ianotto J, Chauveau A, Boyer-Perrard F, Gyan E, Kiladjian J. LongTerm Outcome of Patients with MPN-Associated Myelofibrosis Treated with Peg-Interferon-α2a, a French Intergroup of Myeloproliferative Neoplasms (FIM) Study ASH. 2017;634:A323.
70. Bose P, Daver N, Pemmaraju N, Jabbour E, Verstovsek S. Sotatercept (ACE-011) alone and in combination with Ruxolitinib in patients (pts) with myeloproliferative neoplasm (MPN)-associated myelofibrosis (MF) and Anemia. ASHA. 2017;634:A255.
71. Gerds A, Tauchi T, Ritchie E, Deininger M, Jamieson C, Talpaz M. Phase I/II Trial of Glasdegib in Heavily Pre-Treated Patients with Primary or Secondary Myelofibrosis. ASH. 2017;634:A258.
72. Gangat N, Swords R, Stein B, et al. A multicenter, open-label, pilot study of alisertib (MLN8237), a novel inhibitor of aurora kinase a, in myelofibrosis. ASHA. 2017;634:A1631.
73. Patnaik M, Gupta V, Schiller G, et al. Results from ongoing phase 1/2 trial of SL-401 in patients with myeloproliferative neoplasms including chronic Myelomonocytic leukemia and primary myelofibrosis. ASHA. 2017;634:A2908.
74. Pemmaraju N, Carter B, Kantarjian H, et al. Interim phase 2 clinical trial results for LCL161, an oral Smac mimetic, in patients with intermediate or high risk myelofibrosis. ASHA. 2017;634:A256.
75. Bose P, Pemmaraju N, Schroeder K, Ferrajoli A, Jabbour E, Verstovsek S. Phase 2 study of Pracinostat in combination with Ruxolitinib in patients (pts) with myelofibrosis (MF). ASHA. 2017;634:A1632.
76. Couban S, Benevolo G, Donnellan W, Cultrera J, S. K, Passamonti F. Phase 1b Results of a Study to Assess the Efficacy and Safety of Vis-modegib in Combination with Ruxolitinib in Patients with Intermediate- or High-Risk Myelofibrosis. ASHA. 2017;624:A4179.
77. Rambaldi A, Lurlo A, Vannucchi A, et al. A two-part study of Givinostat in patients with polycythemia Vera: the maximum tolerated dose selection and the proof of concept final results. ASHA. 2017;634:A253.
78. Mascarenhas J, Lu M, E V, et al. Open Label Phase I Study of Single Agent Oral RG7388 (idasanutlin) in Patients with Polycythemia Vera and Essential Thrombocythemia. ASHA. 2017;634:A254.
79. Holmström MO, Riley CH, Skov V, Svane IM, Hassebalch HC, Anderson MH. Spontaneous T-cell responses against the immune checkpoint PD-L1 in patients with chronic myeloproliferative neoplasms correlate with disease stage and clinical response. J OncoImmunology. 27 2 2018;7(6):e1433521 10.1080/2162402X.2018.1433521

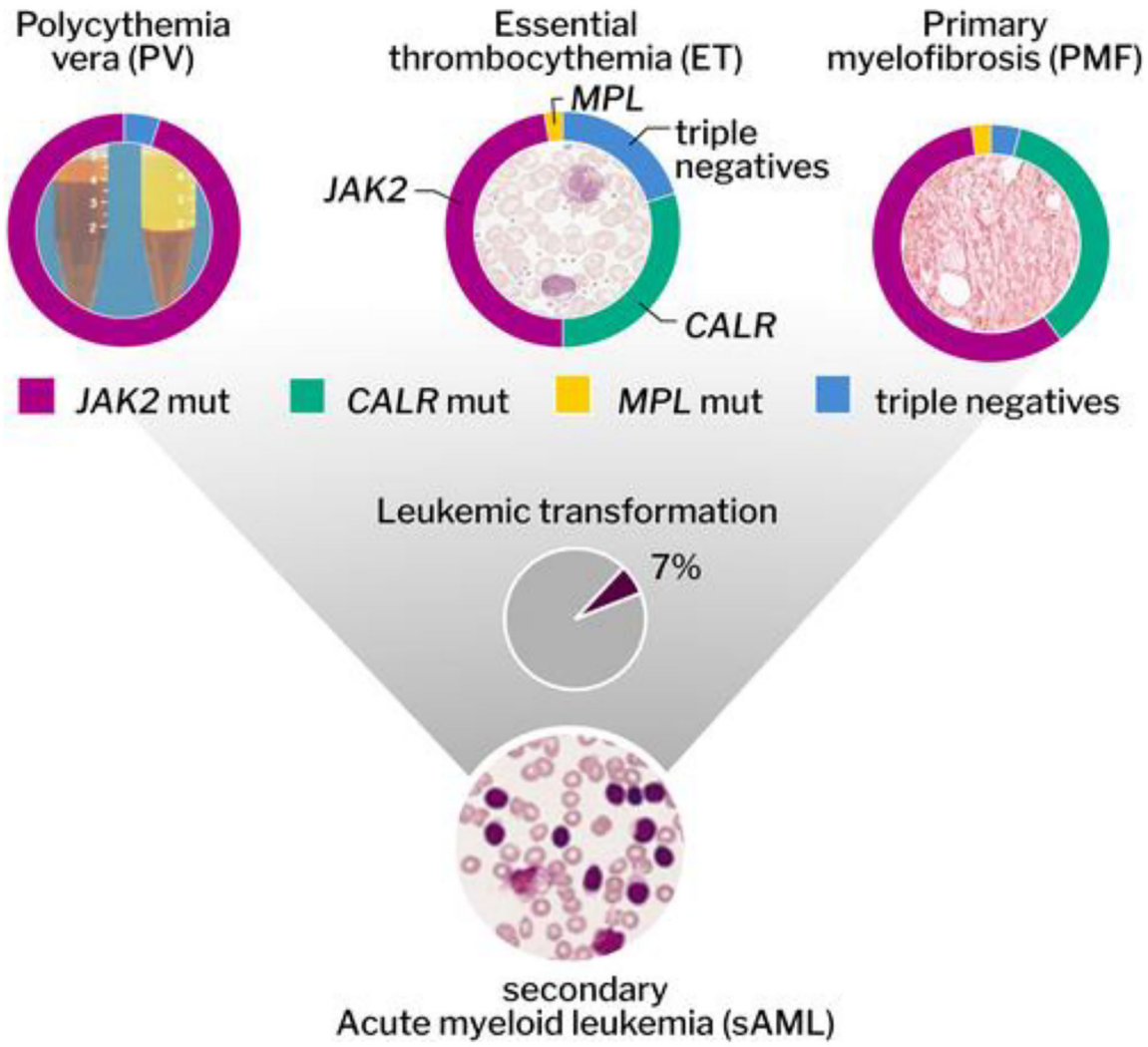

FIGURE 1.
Driver mutations in BCR-ABL1-negative myeloproliferative neoplasms

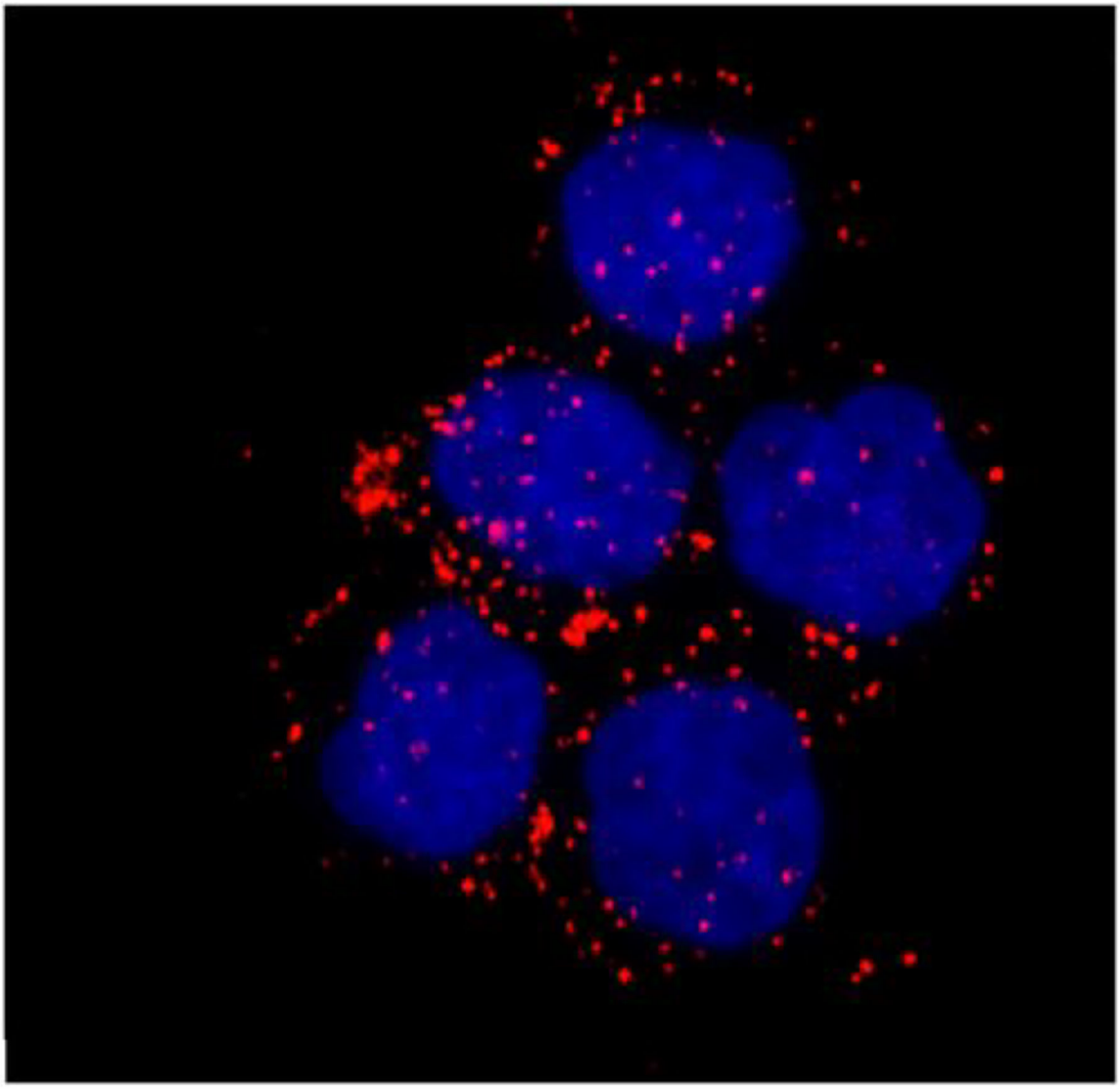

FIGURE 2.

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

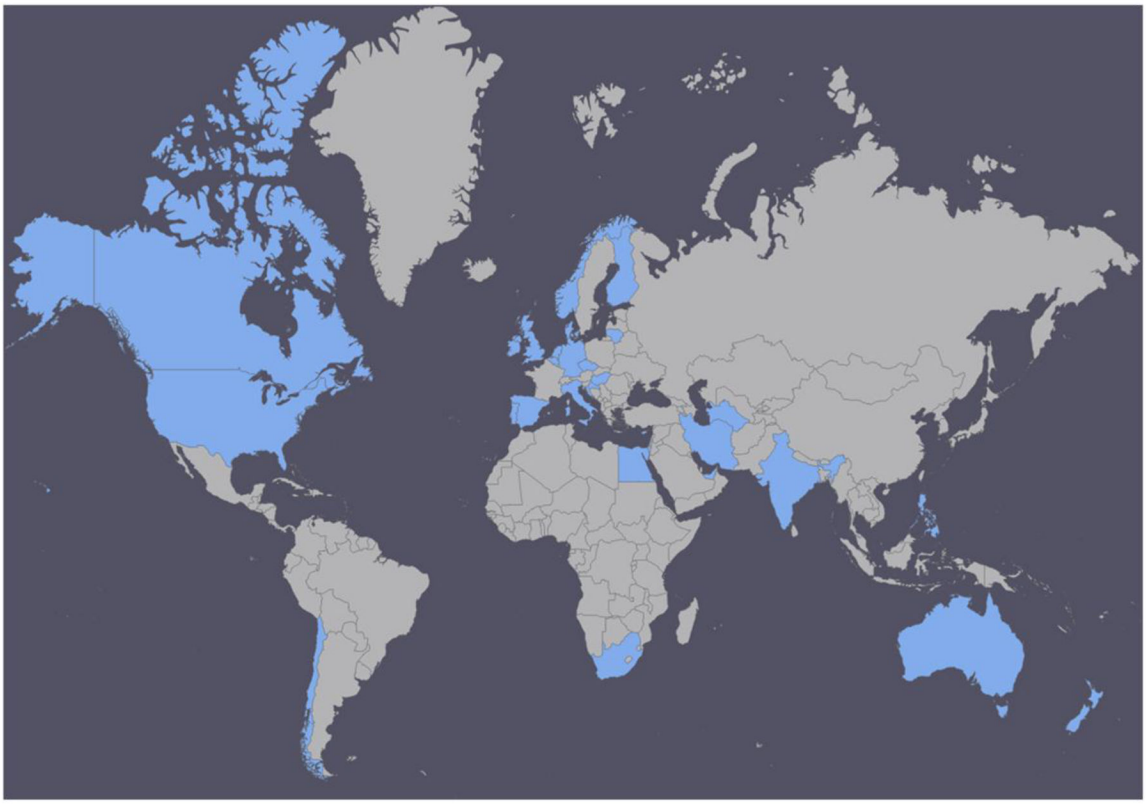

FIGURE 3.
Countries involved in the NUTRIENT Survey in February 2017

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]