EDITORIAL

Myelofibrosis, JAK2 inhibitors and erythropoiesis

Leukemia (2013) 27, 1219–1223; doi:10.1038/leu.2013.72

The term myeloproliferative disorders was introduced by William Dameshek¹ in 1951 to describe four different diseases with a related pathogenesis: polycythaemia vera (PV), essential thrombocythaemia (ET), primary myelofibrosis (PMF) and chronic myeloid leukaemia. The discovery of the Philadelphia chromosome and the fusion gene *BCR-ABL* has permitted the definition of chronic myeloid leukaemia as a specific entity. The pathogenesis of the other non-*BCR-ABL* myeloproliferative disorders, now called myeloproliferative neoplasms (MPNs),² remained elusive until 2005. At that time, these disorders were considered orphan disorders and were not the subject of intensive clinical interest. Nevertheless, it was clear that PV, ET and PMF had closely related pathogeneses, with some clinical, histological and biological features in common. This was underscored by the progression of some ETs to PVs, and some ETs or PVs to myelofibrosis, respectively called post-ET or post-PV myelofibrosis.

The discovery of the JAK2V617F mutation with a high incidence in these three disorders has been a breakthrough in the knowledge of MPN pathogenesis, leading to the demonstration that the three diseases were clonal signalling disorders of the cytokine receptor/JAK2 pathway, and closely related at the molecular level.^{3–6} The JAK2V617F discovery also shed light on disorders that had remained disregarded for a long period. Subsequently, other mutations targeting signalling molecules of the same pathway, such as JAK2 (JAK2 exon 12), the thrombopoietin receptor (MPL) with substitutions at amino acid 515 (W515), LNK and Cbl, were identified, further supporting the hypothesis that MPNs were signalling disorders.^{7–9} In line with chronic myeloid leukaemia, in which the *BCR-ABL* fusion protein may explain the entire disease (from initiation to clinical disease and finally progression towards acute leukaemia), it was tempting to suggest that JAK2V617F was having a similar role in MPN. However, in contrast to *BCR-ABL*, JAK2V617F is associated with three different disorders that may be explained by the oncogenic properties of JAK2V617F.

Unlike *BCR-ABL* or other fusion proteins implicating JAK2, such as TEL-JAK2 or PCM1-JAK2, JAK2V617F is unlikely to induce pathogenic signalling alone, but requires cytokine receptors as a scaffold, which further increases the diversity in the effects of the mutation. Homodimeric type I cytokine receptors, such as EPOR, MPL and G-CSFR, have a central role in the development of myeloproliferative disorders. However, the other cognate cytokine receptors of JAK2V617F, such as the type I (the beta chain of interleukin (IL) 3, granulocyte-macrophage colony-stimulating factor and IL5 receptors, and gp130) and heterodimeric type II receptors (the interferon- γ receptor), are also likely to have a role.¹⁰ This could explain how a single mutation can induce different disorders. Furthermore, in line with murine models, the presence of 9pLOH in PV leading to a second JAK2V617F copy and clinical features have suggested that JAK2V617F disorders are related to the intensity of signalling, with ET arising from a mutation on one allele and PV from a mutation on two alleles.^{11–14} In line with this assumption, the development of myelofibrosis may correspond to an acceleration of the disease with either an important increase in the JAK2V617F clone and/or the acquisition of new mutations, including mutations in signalling molecules,

which will alter differentiation in the megakaryocyte and other lineages.

However, troublesome evidence has suggested that JAK2V617F alone could not be the sole molecular pathogenesis of *BCR-ABL*-negative MPNs. Notably, a significant fraction of acute leukaemia developing from JAK2V617F MPNs was shown to be JAK2WT, and a low burden of JAK2V617F was found in some clonal MPNs, demonstrating that all cells do not bear the mutation.^{15,16} These observations led to the hypothesis of a pre-JAK2 event.

Using whole-genome approaches, a large number of mutations in genes involved in two main pathways were subsequently identified in MPNs. The first pathway concerns epigenetics, either DNA methylation (TET2, DNMT3A and IDH1/2) or the PRC2 complex (EZH2 and ASXL1). The second pathway involves splicing factors (SRSF2, SF3B1 and U2AF35). However, none of these mutations are restricted to MPNs because they are present in a large spectrum of haematological malignancies, particularly in MDS, chronic myelomonocytic leukaemia and mastocytosis, but also in some T cell lymphomas, particularly in angioimmunoblastic T cell lymphoma.^{7–9}

This identified a new key question. What is the need for these different mutations? First, there is strong evidence that mutations in JAK2 and MPL are sufficient to induce myeloproliferation, as demonstrated through analyses of different mouse models¹⁷ and some inherited human disorders. An increasing number of mutations in JAK2, such as JAK2V617I, where a valine at codon 617 is substituted to an isoleucine (JAK2V617I),¹⁸ or in MPL, such as MPLW515R, is observed in inherited thrombocytosis, further demonstrating that the signalling defect is the driving event in these disorders. However, both murine models and inherited human diseases are polyclonal, raising the following question: can a clonal disorder arise from JAK2V617F alone? If it is the case, it is expected that JAK2V617F will give a proliferative advantage to haematopoietic stem cells. However, depending on the mouse model, JAK2V617F provides only a small competitive advantage to haematopoietic stem cells, and sometimes a disadvantage owing to the excessive production of reactive oxygen species.^{19–21}

In PV, the majority of haematopoietic stem cells and progenitors remain JAK2WT for a long period, and unexpectedly for a stem cell disorder, the clonal dominance is acquired during late differentiation by a competitive advantage mediated through the cytokine response.^{12,22} However, early in the disease development, the mutant JAK2V617F haematopoietic stem cell has to outcompete $\sim 1 \times 10^6$ haematopoietic stem cells before the emergence of clinical disease. Mutations occurring in *TET2* and *DNMT3A*,^{23–25} and most likely in other genes yet to be identified, may facilitate this clonal dominance by modifying the biology of haematopoietic stem cells. It remains possible, however, that JAK2V617F provides an advantage to haematopoietic stem cells in pathological conditions such as inflammation or during aging.²⁶ Whether other genetic events are absolutely required to induce the clonal dominance of the JAK2V617F clone remain unclear. The team of T Green²⁷ has recently suggested that the differences between PV and ET are related to the occurrence of a genetic event that promotes the expansion of the homozygous JAK2V617F subclone.²⁷ This possible requirement of another genetic event and the order of mutation acquisition might have a major impact on the development of targeted therapies in the future. Second, PMF is certainly a different disorder than PV and ET, because it requires both clonal dominance and differentiation defects,

particularly in the megakaryocyte and granulo-monocytic lineages. Recent genomic studies in PMF have reinforced this hypothesis by showing the presence of multiple mutations in the same patient.^{7,9} In some cases, three types of mutations might be simultaneously present, one on signalling molecules, one or two on epigenetic regulators and one on splicing molecules. Thus, PMF is an extremely heterogeneous disorder and might be closely related to other myeloproliferative/myelodysplastic disorders, such as chronic myelomonocytic leukaemia. It is still unknown if post-PV or post-ET myelofibrosis is identical to PMF, although it is expected that mutations in signalling molecules might be more important in the pathogenesis of these types of myelofibrosis. Overall, the situation in humans might be different from mouse models, in which strong signalling through MPL is sufficient to induce a disorder mimicking PMF.⁴⁸ Third, other mutations, such as in RUNX1 and P53, are associated with progression to acute leukaemia.

Based on the central role of JAK2 in disease development, targeting the kinase domain of JAK2 is the current approach in the development of new therapies for MPNs. Several small molecules targeting its ATP-binding pocket in its active conformation have been developed and used in clinical trials.^{29,30} One of them (ruxolitinib) has been approved by health authorities for its clinical use.^{31,32} However, none of these molecules, except perhaps LY2784544, have specificity for JAK2V617F. As JAK2 is an indispensable kinase for EPO and TPO signalling, it is expected that efficient JAK2 inhibition will induce anaemia and thrombocytopenia, which, in a clinical setting, is a 'toxic' effect of the molecule. With such an approach, it would be impossible to induce the complete JAK2 inhibition necessary if the goal is to cure the disease. Furthermore, as the present inhibitors stabilize the molecule in an active conformation, JAK2 may heterodimerize with other JAK molecules, such as JAK1 and TYK2, and lead to persistent signalling, which may explain why these molecules are surprisingly well-tolerated.³³ Although all these JAK2 inhibitors exert some level of specificity towards JAK2, they may affect other kinases. For example, ruxolitinib and CYT387 are JAK2 and 1 inhibitors, respectively, whereas SAR 302503 (TG101348) is more specific for JAK2.³⁴ Nevertheless, they may inhibit the kinase activity of other targets, including TYK2 or Aurora, FLT3 and RET, and eventually have off-target effects on non-kinase molecules, which will depend on the drug.³⁴

At present and regardless of the JAK2 inhibitors used, very similar clinical responses are observed in myelofibrosis. They rapidly decrease the spleen size by >30% in a majority of patients and constitutional symptoms such as fatigue, fever, night sweats, pruritus and weight loss.³⁰ In contrast, none have a clear effect on the clonal disorder, although some decrease in the JAK2V617F burden and the intensity of the fibrosis has been described in a few patients. Thus, the most evident benefit is a rapid improvement in myelofibrosis-associated symptoms, which may lead to an increase in overall survival.^{31,35} This benefit is related to an anti-inflammatory effect of the JAK2 inhibitors, as evidenced by the decrease in the plasma level of several inflammatory cytokines. It was suggested that the effects on splenomegaly were also mediated through the inhibition of cytokine production and action.³⁶ However, some more subtle differences are observed depending on the inhibitors used. For example, it has been suggested that the effects of SAR 302503 on the spleen volume and constitutional symptoms could be in part cytokine-independent.³⁷

Overall, it is assumed that JAK2 inhibitors may block the signalling of inflammatory cytokines, such as the IL6 family, or the interferon γ response, but how they inhibit the synthesis and release of inflammatory cytokines remains unknown. The target cells (monocytes, a subset of lymphocytes, dendritic cells, megakaryocytes) for such inhibitory effects are also unknown. As these effects on inflammation might be predominant with JAK2/

JAK1 inhibitors, some companies are developing pure JAK1 inhibitors to have a more specific effect on inflammation without the 'toxic' effects on erythropoiesis and megakaryopoiesis mediated by JAK2 inhibition. Overall, the present approach is not to attempt to cure the disease, but mainly to decrease the symptoms of the disease, improve quality of life, extend the chronicity of the disorder and perhaps prevent some acute transformation. Other totally unexpected differences have also been observed among the inhibitors in clinical trials, particularly effects on haematological parameters, such as anaemia and thrombocytopenia.

In this issue, Pardanani *et al.*³⁸ report the effect of a new JAK1 and JAK2 inhibitor, CYT387, in patients with high or intermediate-risk primary and secondary myelofibrosis. The authors found that the inhibitor had the same effects as other inhibitors on the constitutional symptoms and spleen volume; unexpectedly, a marked improvement in red cells was observed in 70% of transfusion-dependent patients that became transfusion-independent for more than 12 months. To demonstrate that the patients were truly transfusion-dependent, the authors used very stringent criteria, as the consensus definition of red blood cell transfusion dependency recently changed.³⁹ The data clearly show that the treatment greatly improved the anaemia.

Anaemia is a frequent symptom in myelofibrosis and is observed in ~35% of patients. It is considered as a major adverse prognostic factor. The precise mechanism of this anaemia is poorly understood, except at the end of the disease when associated with profound cytopenia, and it appears multifactorial. The low haematocrit can partially be related to haemodilution due to the increased spleen volume along with other metabolism alterations. Presently, this parameter is difficult to investigate, as the measurement of red cell mass and plasma volume is not easily and routinely available. However, the major mechanism is related to a defect in erythropoiesis, and several intermingled mechanisms may combine to induce anaemia, which are as follows: (1) In humans, it has been suggested that spleen erythropoiesis is in large part ineffective, possibly due to the specific spleen microenvironment. In addition, transforming growth factor- β 1 has a central role in myelofibrosis development, and is present in an active form in the marrow and spleen environment. Transforming growth factor- β 1 may decrease the production of red blood cells by accelerating differentiation and inhibiting proliferation.⁴⁰ Other molecules of the transforming growth factor beta family, such as activin A, and GDF members, such as GDF11, may also inhibit erythropoiesis. Targeting these molecules might be an interesting strategy to correct the anaemia.⁴¹ (2) In PMF, the presence of several mutations in epigenetic regulators and splicing molecules, which can affect erythropoiesis, has been shown. The best example is mutations affecting SF3B1, which lead to ring sideroblasts in MDS and myelofibrosis.^{42,43} However, other more frequent mutations such as TET2 may also induce ineffective erythropoiesis because TET2-knocked out mice display an excess of immature erythroblasts, with a decrease in haematocrit.²⁴ These mutations in epigenetic regulators or splicing molecules may also affect the synthesis of molecules involved in the erythrocyte structure, explaining the frequent presence of haemolysis. Overall, ineffective erythropoiesis, as in β -thalassaemia, for example, may profoundly modify iron metabolism and increase the iron absorption through the low level of hepcidin. It has been shown that erythrocytes from β -thalassaemia patients release a high level of GDF15, which inhibits hepcidin synthesis.⁴⁴ Recently, a similar defect has been shown in patients with SF3B1 mutations in MDS.⁴⁵ However, it is not yet known if such a mechanism is also operating in anaemic patients with myelofibrosis. (3) The systemic inflammation present in myelofibrosis is considered one of the major mechanisms of the associated chronic anaemia. The mechanism of inflammatory anaemia is primarily related to a sequestration of iron in macrophages through increased levels of

hepcidin, inhibiting iron export and subsequent erythrocyte iron uptake by ferroportin degradation.⁴⁶ The increased hepcidin levels are related to inflammatory cytokines through IL1, IL6 or IL22. Furthermore, other inflammatory cytokines, such as TNF alpha and interferon- γ , in addition to increased apoptosis of erythroid progenitors and precursors, may increase iron uptake by macrophages.⁴⁶ This mechanism appears in contrast to the previous one. However, the mechanisms can be complementary if GDF15 inhibits the synthesis of hepcidin in hepatocytes, but not in macrophages, allowing excess iron absorption and a decrease in iron availability for erythropoiesis, which results in a functional iron deficiency. (4) Finally, the anaemia can be worsened by blood loss related to thrombocytopenia or other mechanisms.

By acting at different levels, JAK2 inhibitors may have both detrimental and favourable effects on erythropoiesis. The most evident effect is that they strongly inhibit erythroid differentiation by acting on survival and proliferation, because JAK2 activity is indispensable for EPO signalling. The induction of anaemia is a common feature of JAK2 inhibition and the consequence of efficient JAK2 inhibition. However, in a β -thalassaemia mouse model, it has been shown that JAK2 inhibitors can decrease ineffective erythropoiesis by inducing apoptosis of the more pathological erythroid precursors, diminishing the splenomegaly, and thus inducing a more efficient erythropoiesis.^{47,48} It is possible that such a mechanism may partially explain the decrease in spleen volume and some improvement of erythropoiesis after JAK2 inhibition in myelofibrosis. Nevertheless, the anti-inflammatory effects of JAK2 inhibitors may be the most likely mechanisms for explaining the anaemia response. It has also been suggested that immunomodulatory drugs, such as lenalidomide and pomalidomide, may correct the anaemia in myelofibrosis through an anti-inflammatory mechanism.⁴⁹ Here, in line with this hypothesis, the investigators found a decrease in circulating IL-1 β and IL-1RA levels that correlate with red blood cell transfusion independence.³⁸ In addition, JAK2 inhibition may have other targets, such as some G-protein-coupled receptors (GPCRs). Angiotensin II, an important mediator of blood pressure and volume, may also regulate erythropoiesis in EPO-dependent and -independent manners.^{50,51} The main receptor of angiotensin II, AT1 (a GPCR), can also activate JAK2, but the precise role of JAK2 in its signalling remains unknown.⁵²

Having observed the contrasting effects of JAK2 on pathological erythropoiesis, we can speculate that the effects of JAK2 inhibition

can be quite different depending on the level and kinetics of JAK2 inhibition and its effects on JAK1 (Figure 1). For example, a powerful JAK2 inhibitor will induce anaemia. In this situation, the inhibition of erythropoiesis would be predominant. In contrast, a weak JAK2 inhibitor may correct anaemia in myelofibrosis if, in addition, it has a powerful inhibitory effect on JAK1. In this situation, its predominant effects will be an improvement of ineffective erythropoiesis and a relief of inflammation-altered erythropoiesis. Furthermore, it remains possible that the efficacy of JAK2 inhibitors might be dependent on the type of JAK2/cytokine receptor complex. A molecule might have low inhibitory activity on a JAK2/cytokine receptor complex, and in contrast, a high activity on a GPCR/JAK2 complex. These hypotheses may explain the differences observed in haematological parameters between the different JAK inhibitors presently used in clinical trials. They suggest that CYT387 only partially inhibits the JAK2 activity mediated through EPOR due to its intrinsic activity or pharmacokinetics, whereas it may have a strong inhibitory effect on JAK1. The CYT387-specific side effects on blood pressure (hypotension and hypertension) might be related to an efficient inhibitory activity of a JAK2/GPCR complex. However, we cannot exclude the contributions of off-target effects to these differences.

As the JAK2 inhibitors are presently used primarily to improve myelofibrosis-associated symptoms, different types of inhibitors can have a role in treatment. CYT387 might be used in patients with anaemia. A similar concept can be extended to thrombocytopenia. JAK2 has a central role in MPL signalling, but TYK2 in the presence of JAK2 (heterodimers?) can also induce signalling.^{33,53} Thus, it is possible that a JAK2 inhibitor without significant activity on TYK2, such as SAR 302503, may induce moderate thrombocytopenia. An important point is that depending on the type of inhibition achieved by a JAK2 inhibitor, combination treatments may or may not be indicated. For example, if CYT387 mainly acts as an anti-inflammatory agent and an inhibitor of GPCR/JAK2 complexes, and less as a cytokine receptor/JAK2 inhibitor, then its association with immunomodulatory molecules might not lead to synergic results.

Can we go further with JAK2 inhibition than treating the associated symptoms, providing great benefits for patients? Achievement of this will require the development of more specific inhibitors for JAK2V617F or the association of JAK2 inhibitors with different signalling inhibitors that specifically inhibit JAK2 constitutive activation, such as HSP90 or PI3 kinase inhibitors.^{54,55}

Figure 1. Model of the mechanisms explaining the different effects of JAK2 inhibitors on red cell production in myelofibrosis.

Such approaches might be more efficient in PV or ET, which are essentially dependent on constitutive JAK2 inhibition, than in PMF, where several pathways are simultaneously affected in the majority of cases.

The emergence of kinase inhibitors has shed light on the pathogenesis of MPN, but much remains to be discovered to improve the outcome and quality of life of patients affected by MPN, and also for our understanding of the molecular and cellular mechanisms involved in MPN. Close collaboration between clinicians and basic scientists will certainly allow us to reach these goals.

ACKNOWLEDGEMENTS

We are grateful to F Wendling, S Constaninescu, O Hermine, N Casadevall and I Plo for their improvements to the manuscript. WV is a member of the 'équipe labellisée' by the Ligue contre le cancer 2013, headed by H Raslova.

W Vainchenker^{1,2,3,4} and F Favale^{1,2,3}

¹INSERM UMR1009, Institut Gustave Roussy, Villejuif, France;

²Institut Gustave Roussy, Villejuif, France;

³Université Paris-Sud, Institut Gustave Roussy, Villejuif, France and

⁴Laboratory of Excellence GR, Institut Gustave Roussy, Villejuif, France

E-mail: verpre@igr.fr

REFERENCES

- Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood* 1951; **6**: 372–375.
- Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 2008; **22**: 14–22.
- Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S *et al*. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; **365**: 1054–1061.
- James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C *et al*. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; **434**: 1144–1148.
- Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR *et al*. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779–1790.
- Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ *et al*. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005; **7**: 387–397.
- Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and pathogenesis of myeloproliferative neoplasms. *Blood* 2011; **118**: 1723–1735.
- Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia* 2010; **24**: 1128–1138.
- Godfrey AL, Green AR. Genotype-phenotype interactions in the myeloproliferative neoplasms. *Hematol Oncol Clin North Am* 2012; **26**: 993–1015.
- Vainchenker W, Dusa A, Constantinescu SN. JAKs in pathology: role of Janus kinases in hematopoietic malignancies and immunodeficiencies. *Semin Cell Dev Biol* 2008; **19**: 385–393.
- Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. *Blood* 2006; **108**: 2435–2437.
- Dupont S, Masse A, James C, Teyssandier I, Lecluse Y, Larbret F *et al*. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood* 2007; **110**: 1013–1021.
- Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J *et al*. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood* 2008; **111**: 3931–3940.
- Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol* 2002; **30**: 229–236.
- Theocharides A, Boissinot M, Girodon F, Garand R, Teo SS, Lippert E *et al*. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. *Blood* 2007; **110**: 375–379.
- Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ *et al*. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. *Blood* 2006; **108**: 3548–3555.
- Li J, Kent DG, Chen E, Green AR. Mouse models of myeloproliferative neoplasms: JAK of all grades. *Dis Model Mech* 2011; **4**: 311–317.
- Mead AJ, Rugless MJ, Jacobsen SE, Schuh A. Germline JAK2 mutation in a family with hereditary thrombocytosis. *N Engl J Med* 2012; **366**: 967–969.
- Skoda RC. JAK2 impairs stem cell function? *Blood* 2010; **116**: 1392–1393.
- Li J, Spensberger D, Ahn JS, Anand S, Beer PA, Ghevaert C *et al*. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood* 2010; **116**: 1528–1538.
- Mullally A, Poveromo L, Schneider RK, Al-Shahrour F, Lane SW, Ebert BL. Distinct roles for long-term hematopoietic stem cells and erythroid precursor cells in a murine model of Jak2V617F-mediated polycythemia vera. *Blood* 2012; **120**: 166–172.
- James C, Mazurier F, Dupont S, Chaligne R, Lamrissi-Garcia I, Tulliez M *et al*. The hematopoietic stem cell compartment of JAK2V617F-positive myeloproliferative disorders is a reflection of disease heterogeneity. *Blood* 2008; **112**: 2429–2438.
- Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS *et al*. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 2012; **44**: 23–31.
- Quivoron C, Couronne L, Della Valle V, Lopez CK, Plo I, Wagner-Ballon O *et al*. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell* 2011; **20**: 25–38.
- Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C *et al*. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* 2011; **20**: 11–24.
- Fleischman AG, Aichberger KJ, Luty SB, Bumm TG, Petersen CL, Doratotaj S *et al*. TNFalpha facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms. *Blood* 2011; **118**: 6392–6398.
- Godfrey AL, Chen E, Pagano F, Ortmann CA, Silber Y, Bellosillo B *et al*. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. *Blood* 2012; **120**: 2704–2707.
- Villeval JL, Cohen-Solal K, Tulliez M, Giraudier S, Guichard J, Burstein SA *et al*. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. *Blood* 1997; **90**: 4369–4383.
- Atallah E, Verstovsek S. Emerging drugs for myelofibrosis. *Expert Opin Emerg Drugs* 2012; **17**: 555–570.
- Tefferi A. Challenges facing JAK inhibitor therapy for myeloproliferative neoplasms. *N Engl J Med* 2012; **366**: 844–846.
- Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF *et al*. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med* 2012; **366**: 799–807.
- Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V *et al*. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* 2012; **366**: 787–798.
- Koppikar P, Bhagwat N, Kilpivaara O, Manshoury T, Adli M, Hricik T *et al*. Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. *Nature* 2012; **489**: 155–159.
- Reddy MM, Deshpande A, Sattler M. Targeting JAK2 in the therapy of myeloproliferative neoplasms. *Expert Opin Ther Targets* 2012; **16**: 313–324.
- Verstovsek S, Kantarjian HM, Estrov Z, Cortes JE, Thomas DA, Kadia T *et al*. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. *Blood* 2012; **120**: 1202–1209.
- Tefferi A. JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. *Blood* 2012; **119**: 2721–2730.
- Pardanani A, Gotlib JR, Jamieson C, Cortes JE, Talpaz M, Stone RM *et al*. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. *J Clin Oncol* 2011; **29**: 789–796.
- Pardanani A, Laborde RR, Lasho TL, Finke C, Bega K, Al-Kali A *et al*. Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. *Leukemia* 2013, e-pub ahead of print 5 March 2013; doi:10.1037/leu.2013.71.
- Gale RP, Barosi G, Barbui T, Cervantes F, Dohner K, Dupriez B *et al*. RBC-transfusion guidelines update. *Leuk Res* 2012; **36**: 659–660.
- Zermati Y, Fichelson S, Valensi F, Freyssié JM, Rouyer-Fessard P, Cramer E *et al*. Transforming growth factor inhibits erythropoiesis by blocking proliferation and accelerating differentiation of erythroid progenitors. *Exp Hematol* 2000; **28**: 885–894.
- Iancu-Rubin C, Mosoyan G, Wang J, Kraus T, Sung V, Hoffman R. Stromal cell-mediated inhibition of erythropoiesis can be attenuated by Sotatercept (ACE-011), an activin receptor type II ligand trap. *Exp Hematol* 2013; **41**: 155–166.e17.
- Lasho TL, Finke CM, Hanson CA, Jimma T, Knudson RA, Ketterling RP *et al*. SF3B1 mutations in primary myelofibrosis: clinical, histopathology and genetic correlates among 155 patients. *Leukemia* 2012; **26**: 1135–1137.

- 43 Patnaik MM, Lasho TL, Hodnefield JM, Knudson RA, Ketterling RP, Garcia-Manero G *et al.* SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. *Blood* 2012; **119**: 569–572.
- 44 Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT *et al.* High levels of GDF15 in thalassaemia suppress expression of the iron regulatory protein hepcidin. *Nat Med* 2007; **13**: 1096–1101.
- 45 Ambaglio I, Malcovati L, Papaemmanuil E, Laarakkers CM, Della Porta MG, Galli A *et al.* Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of SF3B1. *Haematologica* 2013; **98**: 420–423.
- 46 Weiss G, Schett G. Anaemia in inflammatory rheumatic diseases. *Nat Rev Rheumatol* 2012, e-pub ahead of print 13 November 2013; doi:10.1038/nrrheum.2012.183.
- 47 Rivella S. The role of ineffective erythropoiesis in non-transfusion-dependent thalassaemia. *Blood Rev* 2012; **26**(Suppl 1): S12–S15.
- 48 Ginzburg Y, Rivella S. Beta-thalassaemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. *Blood* 2011; **118**: 4321–4330.
- 49 Pardanani A, Begna K, Finke C, Lasho T, Tefferi A. Circulating levels of MCP-1, sIL-2R, IL-15, and IL-8 predict anemia response to pomalidomide therapy in myelofibrosis. *Am J Hematol* 2011; **86**: 343–345.
- 50 Cole J, Ertoy D, Lin H, Sutliff RL, Ezan E, Guyene TT *et al.* Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. *J Clin Invest* 2000; **106**: 1391–1398.
- 51 Fuchs S, Xiao HD, Cole JM, Adams JW, Frenzel K, Michaud A *et al.* Role of the N-terminal catalytic domain of angiotensin-converting enzyme investigated by targeted inactivation in mice. *J Biol Chem* 2004; **279**: 15946–15953.
- 52 Marrero MB, Paxton WG, Schieffer B, Ling BN, Bernstein KE. Angiotensin II signalling events mediated by tyrosine phosphorylation. *Cell Signal* 1996; **8**: 21–26.
- 53 Staerk J, Defour JP, Pecquet C, Leroy E, Antoine-Poirel H, Brett I *et al.* Orientation-specific signalling by thrombopoietin receptor dimers. *EMBO J* 2011; **30**: 4398–4413.
- 54 Weigert O, Lane AA, Bird L, Kopp N, Chapuy B, van Bodegom D *et al.* Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. *J Exp Med* 2012; **209**: 259–273.
- 55 Guglielmelli P, Barosi G, Rambaldi A, Marchioli R, Masciulli A, Tozzi L *et al.* Safety and efficacy of everolimus, a mTOR inhibitor, as single agent in a phase 1/2 study in patients with myelofibrosis. *Blood* 2011; **118**: 2069–2076.