Dynamics of JAK2 V617F allele burden of CD34\(^+\) haematopoietic progenitor cells in patients treated with ruxolitinib

Ruxolitinib has demonstrated splenomegaly reduction and symptom relief in patients with polycythaemia vera (PV) and myelofibrosis (MF) (Harrison et al, 2012; Verstovsek et al, 2012, 2014; Vannucchi et al, 2015) with limited effect on granulocytic JAK2 V617F mutant load (Harrison et al, 2012; Vannucchi et al, 2015). However, the effect of ruxolitinib on the JAK2 V617F allele burden at the progenitor level is still unknown.

We have assessed the effect of ruxolitinib on haematopoietic stem cells (HSCs, CD34\(^+\) CD38\(^-\)), haematopoietic progenitor cells (HPCs, CD34\(^+\) CD38\(^-\)) and granulocytes from seven patients with JAK2 V617F myeloproliferative neoplasms (PV, n = 4; secondary MF, n = 3). HPCs and HSCs were isolated from peripheral blood in all patients. Isolation of cell subpopulations and quantification of JAK2 V617F allele burden was performed in circulating HSCs, HPCs and granulocytes before starting ruxolitinib and after 6 and 12 months of therapy as previously described (Angona et al, 2015). Additional mutations in TET2 (whole coding sequence), DNMT3A (exon 23), TP53 (exons 2–10), ASXL1 (exon 12), SF3B1 (exons 14 and 15), SRSF2 (exon 1) and U2AF1 (exons 2 and 6–7) genes were studied in granulocytes by Sanger sequencing or pirosequencing using a Next Generation Sequencing (NGS) 454 GS Junior platform (Roche Applied Science, Mannhion, Germany). Mutations detected were also analysed by NGS in DNA extracted from granulocytes, HSCs and HPCs after 6 and 12 months of therapy. The study was approved by the Ethics Committee and informed consent was obtained according to the Declaration of Helsinki.

Ruxolitinib was indicated as second-line treatment in all seven patients. Palpable splenomegaly was present in six patients before starting ruxolitinib with a reduction during the treatment in all of them. Most patients had marked improvement in symptoms. Two patients discontinued ruxolitinib after 6 months of treatment, due to severe anaemia (n = 1) and transformation to acute leukaemia (n = 1).

The quantification of JAK2 V617F allele burden in HSCs, HPCs and granulocytes during ruxolitinib therapy is shown in Fig 1. In HSCs, JAK2 V617F allele burden remained stable in five patients (>50% and <50% in three and two patients, respectively), decreased from 54% to 27% and duplicated from 22% to 47% in one patient each (Fig 1A). A reduction of JAK2 V617F allele burden in HPCs was observed after 6 months of therapy in three patients but this reduction was not confirmed at 1 year (Fig 1B). The granulocytic JAK2 V617F allele burden remained stable in all but one patient (Patient 2) in whom a progressive increase, from 37% at baseline to 87% at 12 months, was observed (Fig 1C).

One patient (Patient 4) showed additional mutations in TET2 (p.R1179 fs and p.E1483X). The mutational burden of both TET2 mutations remained stable in HSCs and granulocytes during ruxolitinib treatment (Fig 2). The allele burden of TET2 p.R1179 fs remained stable in HPCs whereas TET2 p.E1483X presented a mild progressive increase from 13% to 33.9%.

To the best of our knowledge, this is the first study evaluating the effect of ruxolitinib on circulating HSCs and HPCs. Our results showed that ruxolitinib had a minimal impact on the mutant JAK2 V617F allele burden in PV and secondary MF patients, both in granulocytes and CD34\(^+\) cell subpopulations, suggesting a minimal inhibition of the bone marrow JAK2 V617F-mutated cells.

It is noteworthy that the six patients with palpable splenomegaly prior to therapy experienced a reduction of the spleen size during treatment in contrast with the absence of JAK2 V617F allele burden variation in bone marrow progenitor cells in all patients. This could be explained by differences in the microenvironment of the bone marrow and the spleen, with bone marrow progenitors being less sensitive to this treatment in contrast to those stem cells present in the spleen. In this regard, Wang et al (2014) recently showed that AZD1480, a JAK1/2/3 inhibitor, may induce apoptosis in myeloproliferative neoplasm progenitor cells in the spleens of MF patients with a subsequent reduction in splenomegaly.

It has been reported that the presence of mutations in additional genes other than JAK2 V617F may play a role in the transformation of chronic phase PV and MF to acute leukaemia (Swierczek et al, 2011; Brecqueville et al, 2012). In our series, one PV patient harboured two mutations in the TET2 gene, without significant mutational burden variation in any of the cell populations during ruxolitinib treatment. In this sense, if ruxolitinib has an effect on HSCs, its role might be different depend on whether JAK2 V617F and TET2 mutations coexist in the same clone or are present in two independent clones. In the first situation, we would expect that ruxolitinib, by inhibiting JAK2, also had some effect on TET2 mutational load. By contrast, if the two mutations are present in different clones, JAK2 inhibition would result in the expansion of the TET2 clone. In addition,
Fig 1. JAK2 V617F allele burden in stem cells (CD34+CD38−), progenitor cells (CD34+, CD38+) and granulocytes from patients with myeloproliferative neoplasms treated with ruxolitinib. (A) Stem cells (CD34+CD38−). (B) Progenitor cells (CD34+CD38+). (C) Granulocytes. PV, polycythaemia vera; MF, myelofibrosis.

Fig 2. TET2 R1179 fs and E1483X mutational burden in stem cells, progenitor cells and granulocytes prior to and during treatment with ruxolitinib in a patient carrying mutations in the TET2 gene.
the order in which JAK2 and TET2 mutations are acquired might influence the response to targeted therapy, with JAK2 V617F-first patients being more sensitive to JAK2 inhibition (Ortmann et al, 2015). Analysis of single colonies for JAK2 and TET2 mutations in Patient 4 showed that JAK2 V617F was acquired before TET2 mutations (data not shown), but no effect was observed on mutant allele load in any of the mutations. This low impact on the mutational load of CD34+ cells suggests that ruxolitinib probably is not able to modify the clonal evolution of the disease.

The main limitation of the present work is the small number of patients evaluated. In addition, we selected HSCs and HPCs considering CD38 and CD34 staining whereas other studies add CD90 and CD45RA to better discriminate between stem and progenitor cells (Majet et al, 2007). Given that CD38 positivity shows a continuous gradient, the double CD38 intermediate+/CD34+ population was excluded from the analysis. It must be pointed out that the quantification of JAK2 V617F allele burden in the CD38 intermediate+/CD34+ population was similar to that observed in HSCs (data not shown), a feature that is consistent with the correct discrimination between HSCs and HPCs according to CD34/CD38 positivity.

In summary, and despite the abovementioned limitations, our data indicate that ruxolitinib had clinical benefit in terms of reduction of the spleen size in spite of a minimal effect on the JAK2 V617F mutant allele burden of HSCs and HPCs.

Acknowledgements

This study was supported by grants from the Ministry of Education and Science of Spain and Instituto de Salud Carlos III FEDER (PI10/01807, PI13/00557, PI13/00393, RD12/0036/0010) and 2014SGR567.

References


Authorship

AA designed the study, collected the data, performed the molecular studies, performed the statistical analysis, analysed and interpreted the results and wrote the paper. AAL designed the study, collected the data, performed the statistical analysis, analysed and interpreted the results, wrote the paper and approved the final version. BB and CB designed the study, interpreted the results, wrote the paper and approved the final version. RL and CFP performed the molecular studies, interpreted the results and approved the final version.

Conflict of interest

No relevant conflict of interest to declare regarding this article.

Anna Angona1,2,3
Alberto Alvarez-Larran1,2,3
Beatriz Bellosillo3,4,5
Raquel Longaron3,4
Concepción Fernández-Rodríguez2,3
Carlos Besses1,3

1Haematology Department, Hospital del Mar, 2Universitat Autònoma de Barcelona, 3Grup de Recerca Clínica Aplicada en Neoplasies Hematològiques-IMIM (Hospital del Mar Medical Research Institute), 4Pathology Department, Hospital del Mar, and 5Universitat Pompeu Fabra, Barcelona, Spain

E-mail: 95967@parcdesalutmar.cat

Keywords: myeloproliferative disorders, progenitor cells, JAK2 V617F, additional mutations, ruxolitinib

Correspondence

© 2015 John Wiley & Sons Ltd, British Journal of Haematology