bjh review

A journey through infectious risk associated with ruxolitinib

Emanuela Sant'Antonio,[1] Massimiliano Bonifacio,[2] Massimo Breccia[3] and Elisa Rumi[4]

[1]Department of Oncology, Division of Haematology, Azienda USL Toscana Nord Ovest, Lucca, [2]Section of Haematology, Department of Medicine, University of Verona, Verona, [3]Division of Cellular Biotechnologies and Haematology, University Sapienza, Roma and [4]Department of Haematology Oncology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy

Summary

Ruxolitinib has proved to be effective for the treatment of patients with myelofibrosis (either primary or secondary) and polycythaemia vera, and its approval led to a significant change in the current treatment algorithm. Despite its efficacy and beyond its well described haematological toxicity, a peculiar immunosuppressive effect emerged as our clinical experience grew, both within and outside of a clinical trial setting. Definite and negative interactions with multiple pathways of the immune system of patients have been reported so far, involving both adaptive and innate immune responses. These pathophysiological mechanisms may contribute to the increased risk of reactivation of silent infections (e.g., tuberculosis, hepatitis B virus and varicella zoster virus) that have been associated with the drug. Even though such infectious events may be fatal or may lead to significant impairment of organ function, compromising the eligibility of patients for an allotransplant procedure, there are no dedicated guidelines that may help us in assessing and managing the risk of developing serious infections. On this basis, our aim for the present work was to review the current knowledge on the pathophysiological mechanisms through which ruxolitinib may exert its immunosuppressive effect, and to illustrate our personal approach to the management of three peculiar clinical scenarios, for which a risk-based algorithm is suggested.

Keywords: ruxolitinib, myelofibrosis, polycythaemia vera, infection, management.

Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are a group of clonal haematopoietic disorders that share a common pathophysiological ground, i.e., the deregulation of JAK-STAT signalling, with the subsequent promotion of a proliferative and pro-survival phenotype (Vainchenker & Kralovics, 2017). This biochemical pathway is deeply involved in haematopoiesis as well as in the development and functioning of the immune system (Yamaoka *et al*, 2004; Ghoreschi *et al*, 2009), as highlighted by the severe combined immunodeficiency syndrome associated with JAK3 deficiency of (Macchi *et al*, 1995). Indeed, MPNs are associated with a hyperinflammatory state and deregulation of immune homeostasis (Lussana & Rambaldi, 2017), and infections are known to be one of the leading causes of morbidity and mortality in MPN. Both a higher risk of events, either bacterial or viral, and an increased risk of death due to infections have been reported in two large Swedish population-based studies (Hultcrantz *et al.*, 2015a,2015b).

The discovery of the hyperactivity of the JAK-STAT pathway in MPN, as a consequence of one of the three driver mutations (*JAK2* V617F, *MPL* W515K/L, *CALR* exon 9 indels), paved the way to the design and subsequent rapid development of JAK inhibitor drugs, one of which, ruxolitinib, entered routine clinical practice in 2011 in the United States and 2015 in Europe. Ruxolitinib works as a type 1 inhibitor for JAK1 and JAK2 proteins, binding to their kinase domains, and targeting both mutated and wild-type JAK (Hobbs *et al*, 2017).

As clinical experience with the use of ruxolitinib increased, both within and outside of a clinical trial setting, the prospect of a drug-induced, impaired immunosurveillance emerged, suggested by an increased risk of infectious complications and several reports of events that sometimes were atypical in terms of aetiology and clinical presentation (Lussana *et al*, 2017; Dioverti *et al*, 2018; Polverelli *et al*, 2018).

Immunological consequences of JAK inhibition

Recent research has focused on deciphering the complex and context-dependent changes that contribute to this immune deregulation, given that MPNs are, themselves, characterized by several immune abnormalities and autoimmunity, and, more broadly, that inflammation may likely play a role in MPN pathogenesis (Barosi, 2014). Indeed, MPNs often co-occur and share common inflammatory pathways with

Correspondence: Emanuela Sant'Antonio, Department of Oncology, Division of Haematology, Ospedale San Luca, via Guglielmo Lippi Francesconi, 55100 Lucca, Italy.
E-mail: santantonioemanuela@gmail.com

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autoimmune diseases, and some clinical experiences suggest that autoimmune disorders may even predispose to MPN (Anderson *et al*, 2009; Kristinsson *et al*, 2010).

Although these data need to be interpreted with caution, becuase they come from single studies on relatively small and heterogeneous cohorts, MPN patients showed an expanded (Thiele *et al*, 1999) and overactivated (Rameshwar *et al*, 2000) monocytic/macrophagic compartment, a significant elevation of myeloid-derived suppressor cells, which negatively interfere with immunosurveillance (Wang *et al*, 2016), and a reduction in total lymphocytes counts, though cytotoxic T lymphocytes were greatly increased (Cervantes *et al*, 2000). Data regarding regulatory T cells (Tregs), which are probably involved in both tumour immune surveillance and anti-infective response, are discordant (Zhao *et al*, 2008; Keohane *et al*, 2015), while mature natural killer (NK) cells have been reported to be functionally defective, with impaired degranulation and killing capacity (Schönberg *et al*, 2015).

JAK inhibitors have been reported to have several pleiotropic influences on both the innate and the adaptive compartment of the immune system, thus delineating the biological ground for the increased incidence of treatment-emergent infections. In detail, ruxolitinib-treated patients showed a clear reduction in circulating, NK cells, probably because the drug interferes with cytokine signals that are crucial for NK terminal maturation [e.g., interleukin (IL) 2 and IL15]. Notably, NK cell deficiency was even more pronounced in those who developed relevant infections (Schönberg *et al*, 2015). Dendritic cells, which have a crucial role as antigen-presenting cells in inducing antigen-specific T-cell responses, are quantitatively reduced and functionally impaired after ruxolitinib exposure, showing defective costimulatory properties, decreased cytokine production and altered migration (Heine *et al*, 2013). The latter is actually due to an off-target, JAK1/JAK2-independent, inhibitory effect on two kinases, namely the Rho-associated coiled-coil kinases 1 and 2 (ROCK1 and ROCK2), which control actin cytoskeleton remodelling and cellular adhesion, migration and trafficking (Rudolph *et al*, 2016). Moreover, *in vitro* exposure to ruxolitinib almost completely blocked differentiation of monocytes into dendritic cells (Heine *et al*, 2013).

In the original phase I/II clinical trial (Verstovsek *et al*, 2010), patients showed a significant expansion of non-classical monocytes that correlated with clinical response, and this change was particularly evident during the first years of therapy (Prijic *et al*, 2015). In addition, recent findings suggest that the drug might impair monocytic superoxide radical formation (Bjorn *et al.*, 2019).

Regarding T lymphocytes, two studies confirmed a rapid, ruxolitinib-induced downregulation of secretion of inflammatory cytokines by CD4+ cells, both *in vitro* and *in vivo* (Keohane *et al*, 2015; Parampalli Yajnanarayana *et al*, 2015) and a significant reduction of Tregs, which control autoreactive lymphocytes and regulate both innate and adaptive immune responses. Notably, in the few patients that had dose reduction or discontinued the drug, Treg levels did not completely recover after up to 8 months off-therapy (Massa *et al*, 2014).

Finally, even though the consequences of JAK inhibitor therapy on B lymphocytes are unclear, their physiological development and activity rely on a complex interaction with other subsets of immune cells, including T lymphocytes and dendritic cells. Therefore, even though the net effect of ruxolitinib on adaptive antibody responses cannot be accurately predicted, their important role in mounting an effective immune response should be kept in mind.

Taken together, all of these data on the immunological consequences of JAK inhibitor drugs give a biological explanation for their successful use in a wide range of immune-mediated diseases, ranging from acute and chronic graft-versus-host disease (Spoerl *et al*, 2014; Zeiser *et al*, 2015; Modi *et al*, 2019), to life-threatening syndromes, such as haemophagocytic lymphohistiocytosis (Das *et al*, 2016; Maschalidi *et al*, 2016; Broglie *et al*, 2017; Sin & Zangardi, 2017).

Dealing with the risk of infection

Recently, the European Conference on Infections in Leukaemia (ECIL) (Maschmeyer *et al*, 2019) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Infections in Compromised Hosts (ESGICH) (Reinwald *et al*, 2018) reviewed the safety profile of a wide range of targeted agents and immunotherapeutic drugs, including ruxolitinib; both papers provided a critical state-of-the-art on infectious complications associated with their use, and suggested some preventive recommendations. As with many other new targeted and/or immunomodulating drugs that are currently in use for the treatment of haematological malignancies, it is now well established that clinicians need to be aware and vigilant of the risk of infections, and need to be especially proactive in treating any episodes. Moreover, a focused screening is mandatory, to be performed at baseline.

However, there are still a few uncertainties that need to be addressed. Given that the immunosuppression linked to ruxolitinib is expected to be durable, clinicians have to face the decision of (i) when it is appropriate to use a long-term, secondary prophylaxis for varicella zoster virus (VZV), and (ii) how to address the risk of hepatitis B virus (HBV) reactivation, with prophylaxis or pre-emptive intervention, ideally following a risk-adapted approach and, finally, (iii) how to effectively screen for latent tubercular infection.

The present work illustrates our personal approach, discussing three clinical scenarios that may be faced by those who care for patients with MPN in their daily clinical practice, with the aim of incorporating some risk-adapted considerations and taking into account very recent, prospective data on HBV reactivation in this setting (Gill *et al*, 2019).

However, we recognize its limitations, as we cannot give solid or evidence-based management recommendations, because the following discussion is based on our clinical expertise and judgment, and on a critical discussion of literature data and guidelines dedicated to immunocompromised patients.

Though their frequency is not negligible, we chose not to include urinary and respiratory tract infections, because their management should follow local and institutional guidelines, regardless of JAK inhibitor treatment; accordingly, we strictly adhere to the recommendations regarding seasonal flu vaccination, which we offer to all myelofibrosis (MF) patients treated with ruxolitinib, regardless of age, and pneumococcal vaccination for those older than 65 years. Furthermore, we encourage discussion with infectious disease specialists regarding meningococcal vaccination, which is generally recommended for patients who are candidates for immunosuppressive or antineoplastic treatments.

Clinical scenario 1, VZV reactivation

A 67-year-old woman was diagnosed with International Prognostic Score System (IPSS) intermediate-2 primary myelofibrosis (PMF) in October 2016; she complained of drenching night sweats and abdominal discomfort due to severe splenomegaly that was palpable 16 cm below the left costal margin. At baseline, she did not report any previous zoster episode, her serological profile for human immunodeficiency virus (HIV), HBV and hepatitis C virus (HCV) was negative, and there were no signs of latent tubercular infection. She was, therefore, eligible for symptomatic treatment with ruxolitinib, which was started in a few weeks. She rapidly experienced an almost complete resolution of her symptoms, and obtained a spleen response, with more than 50% reduction in palpatory splenomegaly after the first 6 weeks of treatment. In February 2017, after about 3 months of ruxolitinib, she called us because she was really concerned about a new onset of a tingling and burning sensation on her left shoulder. On physical examination, a vesicular rash was noted on her left sub-scapular area.

Patients treated with ruxolitinib had a statistically significant increased risk of herpes zoster infection compared to controls, both in the original reports of three trials in polycythaemia vera (PV) (Vannucchi *et al*, 2015; Mesa *et al*, 2017; Passamonti *et al*, 2017), with an odds ratio of 7·39, and in the long-term publications of randomized trials in MF (Verstovsek *et al*, 2015; Harrison *et al*, 2016) and PV (Verstovsek *et al*, 2016), with an odds ratio of 5·20 (Lussana *et al*, 2017). Moreover, herpes zoster was one of the most frequent infectious complications reported in the phase IIIb JUMP study, both in its first report (Al-Ali *et al*, 2016) and in the primary analysis (Al-Ali *et al*, 2017), with an overall incidence of 5·2%.

During its initial phases, VZV infection is controlled by the innate immune system, in particular by NK lymphocytes, with the subsequent development of VZV-specific T cells; in a normal host, after primary infection, VZV enters a lifelong latency state, characterized by low-level viral replication under continuous host immune surveillance (Duncan & Hambleton, 2015).

Even though previously-reported VZV infections associated with ruxolitinib were essentially mild, a recent case report described an event of thoracic herpes zoster associated with VZV meningoencephalitis, suggesting that severe disease manifestations may also occur (Eyal *et al*, 2017). It is crucial, then, that both haematologists and their patients are aware that potentially serious viral infections might develop, keeping a high index of suspicion that is key to an early diagnosis: patients should be thoroughly informed and educated to seek immediate medical attention if signs of cutaneous VZV develop, since treatment should be administered as early as possible, ideally within the first 72 h of the onset of the rash.

Serological screening for identifying prior infection does not add any valuable information on the subsequent risk of reactivation, and it's generally not recommended. Live attenuated herpes zoster vaccination has been traditionally considered to be contraindicated for immunocompromised patients. However, we acknowledge that the vast majority of adult patients will have had varicella in childhood and probably would have a variable, but still appreciable, residual specific cell-mediated immunity: therefore, it would be less likely for them to have significant morbidity from vaccination (Levin *et al*, 2017). So, testing for prior infection to identify seronegative cases may be useful when vaccination with live-attenuated vaccine is considered as an option.

Recently, the safety and immunogenicity of VZV vaccination has been explored and, indeed, confirmed in patients with rheumatoid arthritis, vaccinated before starting JAK inhibitor therapy with tofacitinib (Winthrop *et al*, 2017). Moreover, we may expect that future strategies to prevent VZV reactivation will change, given that a recombinant, adjuvanted, glycoprotein E subunit vaccine has been developed and approved by the US Food and Drug Administration (FDA) and, recently, by the European Medicines Agency (EMA); it can be safely used in immunocompromised adults and has shown, indeed, greater immunogenicity compared to the live-attenuated one (James *et al*, 2018).

At present, however, primary VZV prophylaxis for patients undergoing ruxolitinib treatment is not recommended based on the available data, while secondary prophylaxis may be considered, on a case by case basis, for those experiencing recurrent reactivations or for severe, serious or disseminated VZV infections. The best duration of prophylaxis could not be determined, because the rates of VZV infection did not decrease over time (Lussana *et al*, 2017).

We do not generally stop ruxolitinib during the acute phase of the disease: while the inhibitory consequences on the levels and functions of T lymphocytes and NK cells might be reversible, *in vivo* recovery kinetics cannot be inferred, so we cannot predict whether these changes on the immune system might be clinically meaningful in terms of

improving the outcome of an acute viral episode. Indeed, most of the information on T-cell function comes from *in vitro* analysis, while NK cell counts were sequentially assessed and reported in a single patient, who completely recovered, but was evaluated 3 months off-drug (Schönberg *et al*, 2015). We acknowledge, however, that the decision not to discontinue ruxolitinib during an episode of VZV reactivation may be influenced by the severity of the clinical picture and/or the involvement of sensitive areas, as in herpes zoster ophthalmicus. Although, as previously mentioned, a quick recovery of immunocompetence is a theoretical assumption, the potential benefits of stopping the drug outweigh any other clinical risk when there is ocular or systemic involvement, or when neurological symptoms may suggest meningoencephalitis, although it seems to be quite rare in the setting of MPN patients treated with JAK inhibitors. Herpes zoster ophthalmicus can result in devastating retinal pathology with acute necrosis, so that every attempt must be made to avoid permanent vision loss.

The metabolism of acyclovir does not interfere nor overlap with that of ruxolitinib, so that their association should not be an issue from a clinical point of view. Indeed, the drug is largely excreted in the urine, unmodified, while only a minor fraction is oxidized by alcohol and aldehyde dehydrogenase in the liver.

Our patient received standard treatment with acyclovir for 1 week. We did not opt for antiviral prophylaxis because her clinical presentation was typical and not severe, and no additional risk factors or comorbidities were present. At her last follow-up, she was still on ruxolitinib treatment with ongoing clinical benefit, and without any further infectious complications or VZV reactivation.

Clinical scenario 2, hepatitis B virus reactivation

In January 2017 a 70-year-old man with a 5-year history of PMF was offered treatment with ruxolitinib for a progressive splenomegaly, palpable 12 cm below left the costal margin, and unintentional weight loss. Screening for HCV infection was negative, while he tested positive for hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb); hepatitis B serum antigen (HBsAg) was negative and HBV-DNA was undetectable.

After HBV enters hepatocytes, its viral genome reaches the nucleus, where it is repaired into a covalently closed circular DNA, which represents a persistent reservoir for eventual, subsequent HBV reactivation (Loomba & Liang, 2017). Host response to HBV infection relies on a complex interaction of several immune pathways that include cytokines, above all interferon (IFN)-gamma and tumour necrosis factor (TNF)-alfa, and cells of the innate immune system, such as dendritic cells, which are key in priming and directing the virus-specific T-cell response (Jung & Pape, 2002). Consequently, pharmacological interference with those cytokines, as well as inhibition of the function of T lymphocytes by kinase inhibitors, as happens with ruxolitinib therapy, may lead to a higher HBV replication state (Loomba & Liang, 2017).

The COMFORT I and II trials excluded patients with active viral hepatitis, and no cases of reactivation were noted. Five patients who experienced HBV reactivation have been described so far as case reports, three of which were treated in a clinical trial: a patient enrolled in the Response trial (Kirito *et al*, 2016) and two cases from the JUMP trial (Perricone *et al*, 2017). Three out of five were HBsAg positive (Caocci *et al*, 2014; Kirito *et al*, 2016; Perricone *et al*, 2017); on other was described as an HBV carrier, so he was probably HBsAg positive (Shen *et al*, 2014); only one out of five reactivations developed in a patient who was HBsAg-negative and anti-HBc positive, with an undetectable viral load (Perricone *et al*, 2017). Notably, a clear relationship between ruxolitinib daily dose and both clinical efficacy and HBV viral load was noted in one of these case reports (Caocci *et al*, 2014).

According to dedicated guidelines (Reddy *et al*, 2015), HBsAg-positive candidates should be referred to a specialist for further assessment and appropriate treatment. HBsAg-negative, anti-HBc positive subjects have a variable risk of reactivation according to their complete virological profile, underlying disease and type and/or duration of the immunosuppressive regimen. Ruxolitinib, although not formally included in the classification (Reddy *et al*, 2015), can operationally be attributed to a moderate risk of reactivation, along with other tyrosine kinase and cytokine inhibitors. It is recommended that these patients are tested for serum HBV-DNA: if viraemic, they should be treated similarly to HBsAg-positive patients. In HBsAg-negative, anti-HBc positive subjects with moderate or low risk of HBV reactivation, preemptive therapy, not prophylaxis, is generally recommended. In selected clinical settings, characterised by a long duration of immunosuppression, limited compliance to monitoring or unknown risk of viral reactivation for new drugs, prophylaxis is preferred.

The safety of a strategy based on monitoring and preemptive therapy has been recently confirmed in a prospective cohort of Chinese MPN patients, with occult HBV infection, undergoing ruxolitinib treatment (Gill *et al*, 2019). Liver biochemistry, serological tests and HBV-DNA monitoring were regularly performed: 4 out of 15 patients experienced HBV reactivation (defined as HBV-DNA $\geq$ 10 iu/ml), and all of them were then treated with entecavir, which was successful in preventing any HBV-related hepatic complication. Of note, ruxolitinib could be safely continued.

Prophylaxis with lamivudine has been widely used in patients with haematological neoplasms, particularly in those treated with B-cell depleting agents. Even though it has a favourable safety profile, it is unfortunately associated with a high rate of drug resistance, particularly when it is used for more than 1 year. Previously mentioned guidelines (Reddy *et al*, 2015; Sandherr *et al*, 2015) support the adoption of a risk-adapted approach, so that prophylaxis with lamivudine

may be appropriately suggested for short-term cancer treatment of approximately 4–6 months duration, while in cases of sustained or long-term immunosuppression (expected to last for more than 12 months), it is recommended that drugs with a higher antiviral potency, like entecavir or tenofovir, are chosen.

Given that antiviral prophylaxis in MPN patients who are candidates for ruxolitinib is expected to exceed 1 year and because a strategy of pre-emptive intervention has been prospectively tested (Gill *et al.*, 2019), we can propose a risk-based algorithm for the management for HBV reactivation (Fig 1).

Prophylaxis was not strictly recommended for our patient, but we recognise that the decision between prophylaxis versus pre-emptive treatment may be discussed on a case by case basis. Whatever the choice, monitoring is of utmost importance, during and after immunosuppression, in order to start pre-emptive treatment as soon as possible. Meanwhile, HBV vaccination is an option for seronegative adult candidates for ruxolitinib.

Our patient did not receive antiviral prophylaxis and was closely monitored so that any eventual changes could be timely captured: he is doing well, after 20 months of ruxolitinib treatment, without any signs of HBV reactivation.

Clinical scenario 3, tuberculosis reactivation risk

In June 2017 a 71-year-old man, with a long history of PV, was diagnosed with secondary MF: he presented with unintentional weight loss, early satiety that was probably due to significant splenomegaly (palpable 9 cm below the left costal margin) and moderate anaemia. Bone marrow biopsy was consistent with our clinical hypothesis. A tuberculin skin test was requested as part of the initial screening process: the test result showed a palpable skin induration of 5 mm.

His personal history was negative for known tuberculosis infection and for household contact or exposure to any active case, and he denied prior Bacillus Calmette-Guérin vaccination (vaccination can decrease TST specificity). An IFN gamma release assay (IGRA; QuantiFERON-TB) was clearly positive, so he was referred for infectious disease consultation.

In the natural history of tuberculosis, after an initial phase of bacterial replication and dissemination, viable bacilli are kept under control, both within macrophages and extracellularly within granulomas, as a result of a dynamic balance between pathogens on the one hand and the host immune system on the other. The net result of this balance is asymptomatic latent tuberculosis infection (LTBI) when host responses predominate, and active disease when bacterial replication prevails (Gengenbacher & Kaufmann, 2012).

Two cases of tuberculosis were reported in the extended-phase publication of COMFORT II (Colomba *et al*, 2012; Harrison *et al*, 2016) and five cases occurred in the primary analysis of the phase IIIb JUMP trial (Al-Ali *et al*, 2017). Furthermore, 11 case reports (Lee *et al*, 2013; Hopman *et al*, 2014; Chen *et al*, 2015; Keizer *et al*, 2015; Palandri *et al*, 2015; Shamil *et al*, 2015; Abidi *et al*, 2016; Branco *et al*, 2016; Malkan & Haznedaroglu, 2017; Tsukamoto *et al*, 2018; Lescuyer *et al*, 2019) described 14 patients experiencing mycobacterial infections in patients treated with ruxolitinib outside of a clinical trial, including a case of *Mycobacterium avium* complex (MAC) disease; another case was reported in a retrospective study (Palandri *et al*, 2018). The vast majority

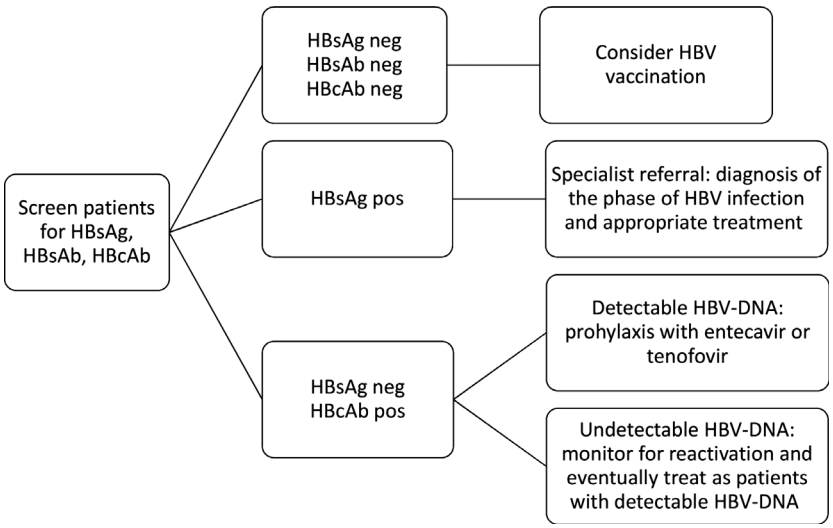

Fig 1. Algorithm for a risk-adapted approach to HBV reactivation. (i) Patients negative for HBsAg and for anti-HBc antibodies should be considered for immunisation; (ii) patients positive for HBsAg should be referred to a specialist for further evaluation, to assess the phase of HBV infection and for choosing the most appropriate treatment; (iii) patients negative for HBsAg and positive for anti-HBcAb should be further tested for HBV DNA; (a) those with a positive viral load should receive prophylaxis, preferably with entecavir or tenofovir; (b) those with a negative polymerase chain reaction should be screened for reactivation on a regular basis, and treated in case. HBcAb, hepatitis B core antibody; HBsAb, hepatitis B serum antibody; HBsAg, hepatitis B serum antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction.

of these mycobacterial infections developed in the absence of specific prophylaxis. Data on the outcome are available for most cases: the infection was fatal in six patients (Keizer *et al*, 2015; Malkan & Haznedaroglu, 2017; Tsukamoto *et al*, 2018; Lescuyer *et al*, 2019), and another one died as a consequence of progressive MF, having discontinued ruxolitinib after mycobacterial infection had been diagnosed (Lee *et al*, 2013). In three cases, ruxolitinib therapy was successfully reinitiated after tuberculosis had been adequately treated (Hopman *et al*, 2014; Palandri *et al*, 2015; Abidi *et al*, 2016); to the best of our knowledge, only one patient (Branco *et al*, 2016) received concomitant anti-tuberculosis drugs and ruxolitinib, unmodified: this association was effective and well tolerated, even though a significant interaction might be predicted because rifampicin is a known, strong cytochrome P450 3A4 (CYP3A4 inducer, thus reducing the half-life of ruxolitinib.

LTBI can be diagnosed by evaluating the response to *in vivo* (tuberculin skin test, TST) or *in vitro* (IGRA; QuantiFERON-TB) stimulation by *Mycobacterium tuberculosis* antigens. TST may suffer from cross-reactivity with environmental mycobacteria, while IGRAs have higher specificity; however, some studies suggest a poor concordance between the two methods (Talati *et al*, 2009). Both tests have poor sensitivity in immunocompromised patients, such as those with haematological malignancies, and none can distinguish between active and latent disease (Anibarro & Pena, 2014).

According to international guidelines (Lewinsohn *et al*, 2017), there are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in immunocompromised patients: it has been suggested that a combined TST-IGRA test is probably the best way to improve sensitivity, particularly when the consequences of missing a case of LTBI exceed the risk of adverse events related to the treatment, mostly liver toxicity (Anibarro & Pena, 2014; Lewinsohn *et al*, 2017).

We usually perform a routine chest X-ray to check for abnormalities consistent with prior tuberculosis, together with either a TST test or QuantiFERON-TB as first-line LTBI assessment; we usually prefer TST for patients that have not been pre-treated with chronic, systemic steroids or with potentially immunomodulating drugs (IFNs, experimental therapies including histone deacetylase inhibitors or different JAK inhibitors), as we can assume that they have a more preserved immune function. Otherwise, we would start with QuantiFERON-TB. However, we acknowledge that institutional guidelines and local feasibility of both tests may vary, so that a thorough discussion with infectious disease specialists is key in addressing this issue.

Even though screening for latent TB is not considered mandatory (Reinwald *et al*, 2018; Maschmeyer *et al*, 2019), we screen every patient who is a candidate for ruxolitinib, because we deem that the risk of missing a patient who may benefit from prophylaxis is not negligible, from a clinical point of view.

Eventually, the patient was diagnosed with LTBI and, being a candidate for ruxolitinib treatment, a 9-month course of isoniazid was prescribed. Once a diagnosis of latent or active disease is made, the treatment should follow the general principles relevant to those in the general population.

Isoniazid metabolism is primarily hepatic through acetylation and subsequent oxidation by the CYP-P450 enzymatic complex, and it may act *in vivo* as a weak to moderate CYP3A4 inhibitor (Desta *et al*, 2001; Wen *et al*, 2002).

Given the risk of liver toxicity, a well-known complication of the drug, caution is needed when combining isoniazid and ruxolitinib: close monitoring is recommended, and it may be prudent and safer to start with a reduced dose of ruxolitinib, that may later be increased, should it be tolerated and clinically appropriate.

While treatment regimens for LTBI are essentially based on a single drug, first-line treatment of active tuberculosis relies on the association of four drugs, namely isoniazid, rifampicin, pyrazinamide and ethambutol, that are all administered for 2 months (so-called "Intensive Phase"), followed by at least 4 months ("Continuation Phase") in which patients only receive isoniazid and rifampicin. At least three of these drugs (all but ethambutol) may cause clinically relevant hepatotoxicity. Although *in vitro* studies failed to show relevant inhibition of hepatic CYP P450 isoforms when each single drug was tested (Cao *et al*, 2017), we believe that, in cases of active infection – either at baseline, or in case of reactivation during JAK inhibitor therapy – the clinical goal is to complete the appropriate plan of anti-tubercular treatment, ensuring adherence, preventing emergence of drug resistance and minimizing the risk of adverse drug reactions that may warrant dose reduction and/or dose interruptions. So, we advise either not to start or to discontinue ruxolitinib in such a clinical scenario.

Conclusions

Over the past few years, there have been significant changes in the therapeutic landscape of MPN, thanks to extensive clinical research in the field of JAK inhibitors that led to the approval of ruxolitinib for MF and PV patients. However, we have learned that the use of this new, targeted agent is not without its hazards, as it interferes with multiple immune pathways that physiologically protect the host and maintain a proper immunosurveillance. Indeed, published data suggest that the infection risk of MPN patients treated with ruxolitinib may be clinically relevant.

A careful assessment of patients' risk is necessary, together with a limited set of screening procedures, but we recognize that this might not be enough to avoid potentially severe, and even fatal, events. We thoroughly assess the infectious risk profile of any individual patient who is a candidate for ruxolitinib, in order to personalize counselling, education and, eventually, prophylaxis. For example, we may discuss the option of an antiviral prophylaxis if a patient has

previously experienced more than one episode of VZV reactivation, or we may refer a patient that has experienced recurrent urinary tract infections for specialist assessment to investigate underlying anatomical or functional abnormalities, and to discuss any prophylactic intervention (either non-antimicrobial or antimicrobial, as per guidelines). In our practice, only a few comorbidities or risk factors may raise eligibility questions for ruxolitinib treatment: HIV infection [though recent research (Gavegnano *et al*, 2017) has suggested that dysregulation of the JAK STAT pathway is associated with viral persistence *in vivo*, and that JAK inhibitors, including ruxolitinib, may favourably impact the magnitude of the HIV reservoir in memory CD4 T cell subsets], active HCV infection in patients that are not candidates for, refuse or fail treatment with direct-acting antivirals, any active infection that is not adequately controlled, history of severe infection requiring intensive care treatment, or history of opportunistic infection that may suggest an underlying immunodeficiency that needs to be addressed or may pose a high risk of morbidity or mortality for the patient.

Given that an evidence-based prophylactic and therapeutic approach is not available, we have shared our personal view, using three clinical scenarios that may be managed in a risk-oriented way.

However, until a solid and effective strategy is available, we strongly suggest close collaboration with infectious diseases specialists and active monitoring for safety, for any patients treated with ruxolitinib, as post-marketing surveillance and case reports will be both fundamental to improve our knowledge of the infectious risk associated with the drug.

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Emanuela Sant'Antonio has no conflict of interest to report. Massimiliano Bonifacio received honoraria from, and has served on speakers' bureaus for, Amgen, Incyte, Pfizer and Novartis. Massimo Breccia received honoraria from Novartis, Pfizer, Incyte and Celgene. Elisa Rumi received consultancy fees from Novartis.

Author contribution

ES conceived the manuscript, reviewed the literature and prepared the first draft. All authors critically revised the article and gave final approval of its final version.

References

Abidi, M.Z., Haque, J., Varma, P., Olteanu, H., Guru Murthy, G.S., Dhakal, B. & Hari, P. (2016) Reactivation of pulmonary tuberculosis following treatment of myelofibrosis with ruxolitinib. *Case Reports in Hematology*, **2016**, 2389038.

Al-Ali, H.K., Griesshammer, M., le Coutre, P., Waller, C.F., Liberati, A.M., Schafhausen, P., Tavares, R., Giraldo, P., Foltz, L., Raanani, P., Gupta, V., Tannir, B., Ronco, J.P., Ghosh, J., Martino, B. & Vannucchi, A.M. (2016) Safety and efficacy of ruxolitinib in an open-label, multicenter, single-arm phase 3b expanded-access study in patients with myelofibrosis: a snapshot of 1144 patients in the JUMP trial. *Haematologica*, **101**, 1065–1073.

Al-Ali, H.K., Foltz, L., Palumbo, G.A., Martino, B., Palandri, F., Liberati, A.M., Le Coutre, P., Garcia-Hernandez, C., Zaritskey, A., Tavares, R., Griesshammer, M., Gupta, V., Raanani, P., Giraldo, P., Bouard, C., Perez Ronco, J., Tiwari, R., Mannelli, F. & Vannucchi, A.M. (2017) Primary analysis of JUMP, a phase 3b, expanded-access study evaluating the safety and efficacy of ruxolitinib in patients with myelofibrosis (N = 2233). *Blood*, **130**, 4204–4204. [Abstract]

Anderson, L.A., Pfeiffer, R.M., Landgren, O., Gadalla, S., Berndt, S.I. & Engels, E.A. (2009) Risks of myeloid malignancies in patients with autoimmune conditions. *British Journal of Cancer*, **100**, 822–828.

Anibarro, L. & Pena, A. (2014) Tuberculosis in patients with haematological malignancies. *Mediterranean Journal of Hematology and Infectious Diseases*, **6**, e2014026.

Barosi, G. (2014) An immune dysregulation in MPN. *Current Hematologic Malignancy Reports*, **9**, 331–339.

Bjorn, M.E., Brimnes, M.K., Gudbrandsdottir, S., Andersen, C.L., Poulsen, H.E., Henriksen, T., Hasselbalch, H.C. & Nielsen, C.H. (2019) Ruxolitinib treatment reduces monocytic superoxide radical formation without affecting hydrogen peroxide formation or systemic oxidative nucleoside damage in myelofibrosis. *Leukemia and Lymphoma*, 1–9. https://doi.org/10.1080/10428194.2019.1579323. [Epub ahead of print]

Branco, B., Metsu, D., Dutertre, M., Marchou, B., Delobel, P., Recher, C. & Martin-Blondel, G. (2016) Use of rifampin for treatment of disseminated tuberculosis in a patient with primary myelofibrosis on ruxolitinib. *Annals of Hematology*, **95**, 1207–1209.

Broglie, L., Pommert, L., Rao, S., Thakar, M., Phelan, R., Margolis, D. & Talano, J. (2017) Ruxolitinib for treatment of refractory hemophagocytic lymphohistiocytosis. *Blood Advances*, **1**, 1533–1536.

Cao, L., Greenblatt, D.J. & Kwara, A. (2017) Inhibitory effects of selected antituberculosis drugs on common human hepatic cytochrome P450 and UDP-glucuronosyltransferase enzymes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, **45**, 1035–1043.

Caocci, G., Murgia, F., Podda, L., Solinas, A., Atzeni, S. & La Nasa, G. (2014) Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. *Leukemia*, **28**, 225–227.

Cervantes, F., Hernandez-Boluda, J.C., Villamor, N., Serra, A. & Montserrat, E. (2000) Assessment of peripheral blood lymphocyte subsets in idiopathic myelofibrosis. *European Journal of Haematology*, **65**, 104–108.

Chen, Y.H., Lee, C.H. & Pei, S.N. (2015) Pulmonary tuberculosis reactivation following ruxolitinib treatment in a patient with primary myelofibrosis. *Leukemia and Lymphoma*, **56**, 1528–1529.

Colomba, C., Rubino, R., Siracusa, L., Lalicata, F., Trizzino, M., Titone, L. & Tolomeo, M. (2012) Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. *BMC Research Notes*, **5**, 552.

Das, R., Guan, P., Sprague, L., Verbist, K., Tedrick, P., An, Q.A., Cheng, C., Kurachi, M., Levine, R., Wherry, E.J., Canna, S.W., Behrens, E.M. & Nichols, K.E. (2016) Janus kinase inhibition

lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. *Blood*, **127**, 1666–1675.

Desta, Z., Soukhova, N.V. & Flockhart, D.A. (2001) Inhibition of cytochrome P450 (CYP450) isoforms by isoniazid: potent inhibition of CYP2C19 and CYP3A. *Antimicrobial Agents and Chemotherapy*, **45**, 382–392.

Dioverti, M.V., Abu Saleh, O.M. & Tande, A.J. (2018) Infectious complications in patients on treatment with ruxolitinib: case report and review of the literature. *Infectious Diseases*, **50**, 381–387.

Duncan, C.J. & Hambleton, S. (2015) Varicella zoster virus immunity: a primer. *Journal of Infection*, **71**, S47–S53.

Eyal, O., Flaschner, M., Ben Yehuda, A. & Rund, D. (2017) Varicella-zoster virus meningoencephalitis in a patient treated with ruxolitinib. *American Journal of Hematology*, **92**, E74–E75.

Gavegnano, C., Brehm, J.H., Dupuy, F.P., Talla, A., Ribeiro, S.P., Kulpa, D.A., Cameron, C., Santos, S., Hurwitz, S.J., Marconi, V.C., Routy, J.P., Sabbagh, L., Schinazi, R.F. & Sekaly, R.P. (2017) Novel mechanisms to inhibit HIV reservoir seeding using Jak inhibitors. *PLoS Path*, **13**, e1006740.

Gengenbacher, M. & Kaufmann, S.H.E. (2012) Mycobacterium tuberculosis: success through dormancy. *FEMS Microbiology Reviews*, **36**, 514–532.

Ghoreschi, K., Laurence, A. & O'Shea, J.J. (2009) Janus kinases in immune cell signaling. *Immunological Reviews*, **228**, 273–287.

Gill, H., Leung, G.M.K., Seto, W.K. & Kwong, Y.L. (2019) Risk of viral reactivation in patients with occult hepatitis B virus infection during ruxolitinib treatment. *Annals of Hematology*, **98**, 215–218.

Harrison, C.N., Vannucchi, A.M., Kiladjian, J.J., Al-Ali, H.K., Gisslinger, H., Knoops, L., Cervantes, F., Jones, M.M., Sun, K., McQuitty, M., Stalbovskaya, V., Gopalakrishna, P. & Barbui, T. (2016) Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. *Leukemia*, **30**, 1701–1707.

Heine, A., Held, S.A., Daecke, S.N., Wallner, S., Yajnanarayana, S.P., Kurts, C., Wolf, D. & Brossart, P. (2013) The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood*, **122**, 1192–1202.

Hobbs, G.S., Rozelle, S. & Mullally, A. (2017) The development and use of janus kinase 2 inhibitors for the treatment of myeloproliferative neoplasms. *Hematology/Oncology Clinics of North America*, **31**, 613–626.

Hopman, R.K., Lawrence, S.J. & Oh, S.T. (2014) Disseminated tuberculosis associated with ruxolitinib. *Leukemia*, **28**, 1750–1751.

Hultcrantz, M., Lund, S.H., Andersson, T.M., Björkholm, M. & Kristinsson, S.Y. (2015a) Myeloproliferative neoplasms and infections; a population-based study on 9,665 patients with myeloproliferative neoplasms diagnosed in Sweden 1987–2009. *Haematologica*, **100**, 666.

Hultcrantz, M., Wilkes, S.R., Kristinsson, S.Y., Andersson, T.M., Derolf, A.R., Eloranta, S., Samuelsson, J., Landgren, O., Dickman, P.W., Lambert, P.C. & Bjorkholm, M. (2015b) Risk and cause of death in patients diagnosed with myeloproliferative neoplasms in Sweden between 1973 and 2005: a population-based study. *Journal of Clinical Oncology*, **33**, 2288–2295.

James, S.F., Chahine, E.B., Sucher, A.J. & Hanna, C. (2018) Shingrix: the new adjuvanted recombinant herpes zoster vaccine. *Annals of Pharmacotherapy*, **52**, 673–680.

Jung, M.C. & Pape, G.R. (2002) Immunology of hepatitis B infection. *Lancet Infectious Diseases*, **2**, 43–50.

Keizer, S., Gerritsen, R., Jauw, Y., Janssen, J., Koopman, B. & Bresser, P. (2015) [Fatal tuberculosis during treatment with ruxolitinib]. *Nederlands Tijdschrift voor Geneeskunde*, **159**, A8650. [Dutch]

Keohane, C., Kordasti, S., Seidl, T., Perez Abellan, P., Thomas, N.S., Harrison, C.N., McLornan, D.P. & Mufti, G.J. (2015) JAK inhibition induces silencing of T Helper cytokine secretion and a profound reduction in T regulatory cells. *British Journal of Haematology*, **171**, 60–73.

Kirito, K., Sakamoto, M. & Enomoto, N. (2016) Elevation of the hepatitis B virus DNA during the treatment of polycythemia vera with the JAK kinase inhibitor ruxolitinib. *Internal Medicine*, **55**, 1341–1344.

Kristinsson, S.Y., Landgren, O., Samuelsson, J., Bjorkholm, M. & Goldin, L.R. (2010) Autoimmunity and the risk of myeloproliferative neoplasms. *Haematologica*, **95**, 1216–1220.

Lee, S.R., Park, Y., Choi, C.W., Kim, S.J., Yhim, H.-Y., Kim, I., Oh, D., Mun, Y.-C., Lim, S.-N., Kim, J.-A., Shin, M.-G. & Won, J.-H. (2013) Efficacy of ruxolitinib in Korean myelofibrosis patients and cases complicated TB lymphadenitis during the treatment. *Blood*, **122**, 1596–1596. [Abstract]

Lescuyer, S., Ledoux, M.P., Gravier, S., Natarajan-Ame, S., Duval, C., Maloisel, F., Mauvieux, L., Toussaint, E., Fornecker, L.M. & Herbrecht, R. (2019) Tuberculosis and atypical mycobacterial infections in ruxolitinib-treated patients with primary or secondary myelofibrosis or polycythemia vera. *International Journal of Infectious Diseases*, **80**, 134–136.

Levin, M.J., Bresnitz, E., Popmihajlov, Z., Weinberg, A., Liaw, K.L., Willis, E. & Curtis, J.R. (2017) Studies with herpes zoster vaccines in immune compromised patients. *Expert Review of Vaccines*, **16**, 1217–1230.

Lewinsohn, D.M., Leonard, M.K., LoBue, P.A., Cohn, D.L., Daley, C.L., Desmond, E., Keane, J., Lewinsohn, D.A., Loeffler, A.M., Mazurek, G.H., O'Brien, R.J., Pai, M., Richeldi, L., Salfinger, M., Shinnick, T.M., Sterling, T.R., Warshauer, D.M. & Woods, G.L. (2017) Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of Tuberculosis in Adults and Children. *Clinical Infectious Diseases*, **64**, 111–115.

Loomba, R. & Liang, T.J. (2017) Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts, management strategies, and future directions. *Gastroenterology*, **152**, 1297–1309.

Lussana, F. & Rambaldi, A. (2017) Inflammation and myeloproliferative neoplasms. *Journal of Autoimmunity*, **85**, 58–63.

Lussana, F., Cattaneo, M., Rambaldi, A. & Squizzato, A. (2017) Ruxolitinib-associated infections: a systematic review and meta-analysis. *American Journal of Hematology*, **93**, 339–347.

Macchi, P., Villa, A., Giliani, S., Sacco, M.G., Frattini, A., Porta, F., Ugazio, A.G., Johnston, J.A., Candotti, F., O'Shea, J.J., Vezzoni, P. & Notarangelo, L.D. (1995) Mutations of JAK-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature*, **377**, 65–68.

Malkan, U.Y. & Haznedaroglu, I.C. (2017) A myelofibrosis case that develops mycobacterial infection after ruxolitinib treatment. *International Journal of Clinical and Experimental Medicine*, **10**, 7304–7307.

Maschalidi, S., Sepulveda, F.E., Garrigue, A., Fischer, A. & de Saint Basile, G. (2016) Therapeutic effect of JAK1/2 blockade on the manifestations of hemophagocytic lymphohistiocytosis in mice. *Blood*, **128**, 60–71.

Maschmeyer, G., De Greef, J., Mellinghoff, S.C., Nosari, A., Thiebaut-Bertrand, A., Bergeron, A., Franquet, T., Blijlevens, N.M.A., Maertens, J.A. & European Conference on Infections in Leukemia (2019) Infections associated with immunotherapeutic and molecular targeted agents in hematology and oncology. A position paper by the European Conference on Infections in Leukemia (ECIL). *Leukemia*, **33**, 844–862

Massa, M., Rosti, V., Campanelli, R., Fois, G. & Barosi, G. (2014) Rapid and long-lasting decrease of T-regulatory cells in patients with myelofibrosis treated with ruxolitinib. *Leukemia*, **28**, 449–451.

Mesa, R., Vannucchi, A.M., Yacoub, A., Zachee, P., Garg, M., Lyons, R., Koschmieder, S., Rinaldi, C., Byrne, J., Hasan, Y., Passamonti, F., Verstovsek, S., Hunter, D., Jones, M.M., Zhen, H., Habr, D. & Martino, B. (2017) The efficacy and safety of continued hydroxycarbamide therapy versus switching to ruxolitinib in patients with polycythaemia vera: a randomized, double-blind, double-dummy, symptom study (RELIEF). *British Journal of Haematology*, **176**, 76–85.

Modi, B., Hernandez-Henderson, M., Yang, D., Klein, J., Dadwal, S., Kopp, E., Huelsman, K., Mokhtari, S., Ali, H., Malki, M.M.A., Spielberger, R., Salhotra, A., Parker, P., Forman, S. & Nakamura, R. (2019) Ruxolitinib as salvage therapy for chronic graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, **25**, 265–269.

Palandri, F., Polverelli, N., Catani, L. & Vianelli, N. (2015) Ruxolitinib-associated tuberculosis: a case of successful ruxolitinib rechallenge. *Annals of Hematology*, **94**, 519–520.

Palandri, F., Tiribelli, M., Benevolo, G., Tieghi, A., Cavazzini, F., Breccia, M., Bergamaschi, M., Sgherza, N., Polverelli, N., Crugnola, M., Isidori, A., Binotto, G., Heidel, F.H., Buccisano, F., Martino, B., Latagliata, R., Spinsanti, M., Kallenberg, L., Palumbo, G.A., Abruzzese, E., Scaffidi, L., Cuneo, A., Cavo, M., Vianelli, N. & Bonifacio, M. (2018) Efficacy and safety of ruxolitinib in intermediate-1 IPSS risk myelofibrosis patients: results from an independent study. *Hematological Oncology*, **36**, 285–290.

Parampalli Yajnanarayana, S., Stubig, T., Cornez, I., Alchalby, H., Schonberg, K., Rudolph, J., Triviai, I., Wolschke, C., Heine, A., Brossart, P., Kroger, N. & Wolf, D. (2015) JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. *British Journal of Haematology*, **169**, 824–833.

Passamonti, F., Griesshammer, M., Palandri, F., Egyed, M., Benevolo, G., Devos, T., Callum, J., Vannucchi, A.M., Sivgin, S., Bensasson, C., Khan, M., Mounedji, N. & Saydam, G. (2017) Ruxolitinib for the treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. *Lancet Oncology*, **18**, 88–99.

Perricone, G., Vinci, M. & Pungolino, E. (2017) Occult hepatitis B infection reactivation after ruxolitinib therapy. *Digestive and Liver Disease*, **49**, 719. [Letter]

Polverelli, N., Palumbo, G.A., Binotto, G., Abruzzese, E., Benevolo, G., Bergamaschi, M., Tieghi, A., Bonifacio, M., Breccia, M., Catani, L., Tiribelli, M., D'Adda, M., Sgherza, N., Isidori, A., Cavazzini, F., Martino, B., Latagliata, R., Crugnola, M., Heidel, F., Bosi, C., Ibatici, A., Soci, F., Penna, D., Scaffidi, L., Aversa, F., Lemoli, R.M., Vitolo, U., Cuneo, A., Russo, D., Cavo, M., Vianelli, N. & Palandri, F. (2018) Epidemiology, outcome, and risk factors for infectious complications in myelofibrosis patients receiving ruxolitinib: a multicenter study on 446 patients. *Hematological Oncology*, **36**, 561–569.

Prijic, S., Manshouri, T., Zhang, Y., Veletic, I., Zhang, X., Bove, J.E., Estrov, Z. & Verstovsek, S. (2015) Ruxolitinib treatment induces a transition of classical to non-classical monocytes in patients with myelofibrosis. *Blood*, **126**, 2838. [Abstract]

Rameshwar, P., Narayanan, R., Qian, J., Denny, T.N., Colon, C. & Gascon, P. (2000) NF-kappa B as a central mediator in the induction of TGF-beta in monocytes from patients with idiopathic myelofibrosis: an inflammatory response beyond the realm of homeostasis. *Journal of Immunology*, **165**, 2271–2277.

Reddy, K.R., Beavers, K.L., Hammond, S.P., Lim, J.K., Falck-Ytter, Y.T. & American Gastroenterological Association Institute (2015) American Gastroenterological Association Institute Guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology*, **148**, 215–219.

Reinwald, M., Silva, J.T., Mueller, N.J., Fortun, J., Garzoni, C., de Fijter, J.W., Fernandez-Ruiz, M., Grossi, P. & Aguado, J.M. (2018) ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Intracellular signaling pathways: tyrosine kinase and mTOR inhibitors). *Clinical Microbiology and Infection*, **24**, S53–S70.

Rudolph, J., Heine, A., Quast, T., Kolanus, W., Trebicka, J., Brossart, P. & Wolf, D. (2016) The JAK inhibitor ruxolitinib impairs dendritic cell migration via off-target inhibition of ROCK. *Leukemia*, **30**, 2119–2123.

Sandherr, M., Hentrich, M., von Lilienfeld-Toal, M., Massenkeil, G., Neumann, S., Penack, O., Biehl, L. & Cornely, O.A. (2015) Antiviral prophylaxis in patients with solid tumours and haematological malignancies–update of the Guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Medical Oncology (DGHO). *Annals of Hematology*, **94**, 1441–1450.

Schönberg, K., Rudolph, J., Vonnahme, M., Parampalli Yajnanarayana, S., Cornez, I., Hejazi, M., Manser, A.R., Uhrberg, M., Verbeek, W., Koschmieder, S., Brummendorf, T.H., Brossart, P., Heine, A. & Wolf, D. (2015) JAK inhibition impairs NK cell function in myeloproliferative neoplasms. *Cancer Research*, **75**, 2187–2199.

Shamil, E., Cunningham, D., Wong, B.L. & Jani, P. (2015) Ruxolitinib associated tuberculosis presenting as a neck lump. *Case Reports in Infectious Diseases*, **2015**, 284168.

Shen, C.H., Hwang, C.E., Chen, Y.Y. & Chen, C.C. (2014) Hepatitis B virus reactivation associated with ruxolitinib. *Annals of Hematology*, **93**, 1075–1076.

Sin, J.H. & Zangardi, M.L. (2017) Ruxolitinib for secondary hemophagocytic lymphohistiocytosis: first case report. *Hematology/Oncology and Stem Cell Therapy*. pii: S1658–3876(17)30090–0. https://doi.org/10.1016/j.hemonc.2017.07.002. [Epub ahead of print].

Spoerl, S., Mathew, N.R., Bscheider, M., Schmitt-Graeff, A., Chen, S., Mueller, T., Verbeek, M., Fischer, J., Otten, V., Schmickl, M., Maas-Bauer, K., Finke, J., Peschel, C., Duyster, J., Poeck, H., Zeiser, R. & von Bubnoff, N. (2014) Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. *Blood*, **123**, 3832–3842.

Talati, N.J., Seybold, U., Humphrey, B., Aina, A., Tapia, J., Weinfurter, P., Albalak, R. & Blumberg, H.M. (2009) Poor concordance between interferon-gamma release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals. *BMC Infectious Diseases*, **9**, 15.

Thiele, J., Kvasnicka, H.M. & Boeltken, B. (1999) Resident bone marrow macrophages in idiopathic (primary) myelofibrosis (IMF): a histochemical and morphometric study on sequential trephine biopsies. *Leukemia Research*, **23**, 983–985.

Tsukamoto, Y., Kiyasu, J., Tsuda, M., Ikeda, M., Shiratsuchi, M., Ogawa, Y. & Yufu, Y. (2018) Fatal disseminated tuberculosis during treatment with ruxolitinib plus prednisolone in a patient with primary myelofibrosis: A case report and review of the literature. *Internal Medicine*, **57**, 1297–1300.

Vainchenker, W. & Kralovics, R. (2017) Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood*, **129**, 667–679.

Vannucchi, A.M., Kiladjian, J.J., Griesshammer, M., Masszi, T., Durrant, S., Passamonti, F., Harrison, C.N., Pane, F., Zachee, P., Mesa, R., He, S., Jones, M.M., Garrett, W., Li, J., Pirron, U., Habr, D. & Verstovsek, S. (2015) Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *New England Journal of Medicine*, **372**, 426–435.

Verstovsek, S., Kantarjian, H., Mesa, R.A., Pardanani, A.D., Cortes-Franco, J., Thomas, D.A., Estrov, Z., Fridman, J.S., Bradley, E.C., Erickson-Viitanen, S., Vaddi, K., Levy, R. & Tefferi, A. (2010) Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *New England Journal of Medicine*, **363**, 1117–1127.

Verstovsek, S., Mesa, R.A., Gotlib, J., Levy, R.S., Gupta, V., DiPersio, J.F., Catalano, J.V., Deininger, M.W., Miller, C.B., Silver, R.T., Talpaz, M., Winton, E.F., Harvey, J.H. Jr, Arcasoy, M.O., Hexner, E.O., Lyons, R.M., Raza, A., Vaddi, K., Sun, W., Peng, W., Sandor, V., Kantarjian, H. & COMFORT-I Investigators (2015) Efficacy, safety, and survival with ruxolitinib in patients with myelofibrosis: results of a median 3-year follow-up of COMFORT-I. *Haematologica*, **100**, 479–488.

Verstovsek, S., Vannucchi, A.M., Griesshammer, M., Masszi, T., Durrant, S., Passamonti, F., Harrison, C.N., Pane, F., Zachee, P., Kirito, K., Besses, C., Hino, M., Moiraghi, B., Miller, C.B., Cazzola, M., Rosti, V., Blau, I., Mesa, R., Jones, M.M., Zhen, H., Li, J., Francillard, N., Habr, D. & Kiladjian, J.J. (2016) Ruxolitinib versus best available therapy in patients with polycythemia vera: 80-week follow-up from the RESPONSE trial. *Haematologica*, **101**, 821–829.

Wang, J.C., Kundra, A., Andrei, M., Baptiste, S., Chen, C., Wong, C. & Sindhu, H. (2016) Myeloid-derived suppressor cells in patients with myeloproliferative neoplasm. *Leukemia Research*, **43**, 39–43.

Wen, X., Wang, J.S., Neuvonen, P.J. & Backman, J.T. (2002) Isoniazid is a mechanism-based inhibitor of cytochrome P450 1A2, 2A6, 2C19 and 3A4 isoforms in human liver microsomes. *European Journal of Clinical Pharmacology*, **57**, 799–804.

Winthrop, K.L., Wouters, A.G., Choy, E.H., Soma, K., Hodge, J.A., Nduaka, C.I., Biswas, P., Needle, E., Passador, S., Mojcik, C.F. & Rigby, W.F. (2017) The safety and immunogenicity of live

zoster vaccination in patients with rheumatoid arthritis before starting tofacitinib: a randomized phase II trial. *Arthritis and Rheumatology*, **69**, 1969–1977.

Yamaoka, K., Saharinen, P., Pesu, M., Holt, V.E. 3rd, Silvennoinen, O. & O'Shea, J.J. (2004) The Janus kinases (Jaks). *Genome Biology*, **5**, 253.

Zeiser, R., Burchert, A., Lengerke, C., Verbeek, M., Maas-Bauer, K., Metzelder, S.K., Spoerl, S., Ditschkowski, M., Ecsedi, M., Sockel, K., Ayuk, F., Ajib, S., de Fontbrune, F.S., Na, I.K., Penter, L., Holtick, U., Wolf, D., Schuler, E., Meyer, E., Apostolova, P., Bertz, H., Marks, R., Lubbert, M., Wasch, R., Scheid, C., Stolzel, F., Ordemann, R., Bug, G., Kobbe, G., Negrin, R., Brune, M., Spyridonidis, A., Schmitt-Graff, A., van der Velden, W., Huls, G., Mielke, S., Grigoleit, G.U., Kuball, J., Flynn, R., Ihorst, G., Du, J., Blazar, B.R., Arnold, R., Kroger, N., Passweg, J., Halter, J., Socie, G., Beelen, D., Peschel, C., Neubauer, A., Finke, J., Duyster, J. & von Bubnoff, N. (2015) Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia*, **29**, 2062–2068.

Zhao, W.B., Li, Y., Liu, X., Zhang, L.Y. & Wang, X. (2008) Involvement of CD4+CD25+ regulatory T cells in the pathogenesis of polycythaemia vera. *Chinese Medical Journal*, **121**, 1781–1786.