

# Accepted Manuscript

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)

Mark Reinwald, Jose T. Silva, Nicolas J. Mueller, Jesús Fortún, Christian Garzoni, Johan W. de Fijter, Mario Fernández-Ruiz, Paolo Grossi, Jose María Aguado

PII: S1198-743X(18)30182-4

DOI: [10.1016/j.cmi.2018.02.009](https://doi.org/10.1016/j.cmi.2018.02.009)

Reference: CMI 1209

To appear in: *Clinical Microbiology and Infection*

Received Date: 10 November 2017

Revised Date: 8 February 2018

Accepted Date: 11 February 2018

Please cite this article as: Reinwald M, Silva JT, Mueller NJ, Fortún J, Garzoni C, de Fijter JW, Fernández-Ruiz M, Grossi P, Aguado JM, 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* (2018), doi: 10.1016/j.cmi.2018.02.009.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**Revised manuscript (CLM-17-12786.R1) [for for AA publication]**

**Review paper**

**Title page**

**Complete title:** 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)

**Authors' affiliations:**

Mark Reinwald<sup>1</sup>, Jose T. Silva<sup>2</sup>, Nicolas J. Mueller<sup>3</sup>, Jesús Fortún<sup>4,5</sup>, Christian Garzoni<sup>6</sup>, Johan W. de Fijter<sup>7</sup>, Mario Fernández-Ruiz<sup>8,5</sup>, Paolo Grossi<sup>9</sup>, Jose María Aguado<sup>8,5</sup>

1. Department of Hematology and Oncology, Klinikum Brandenburg, Medizinische Hochschule Brandenburg Theodor Fontane, Brandenburg an der Havel, Germany
2. Department of Infectious Diseases, University Hospital of Badajoz, Fundación para la Formación e Investigación de los Profesionales de la Salud (FundeSalud), Badajoz, Spain
3. Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland
4. Department of Infectious Diseases, Hospital Universitario "Ramon y Cajal", Madrid, Spain
5. Spanish Network for Research in Infectious Diseases (REIPI RD16/0016), Instituto de Salud Carlos III, Madrid, Spain
6. Departments of Internal Medicine and Infectious Disease, Clinica Luganese, Lugano, Switzerland
7. Department of Medicine, Division of Nephrology, Leiden University Medical Centre, Leiden, The Netherlands
8. Unit of Infectious Diseases, Hospital Universitario "12 de Octubre", Instituto de Investigación Hospital "12 de Octubre" (i+12), School of Medicine, Universidad Complutense, Madrid, Spain
9. Department of Infectious and Tropical Diseases, University of Insubria, Ospedale di Circolo-Fondazioni Macchi, Varese, Italy

- **Word length** (excluding title page, figure legends and references): 9,563

- **Number of figures:** 1
- **Number of tables:** 2
- **Number of references:** 236
- **Funding sources:** This research was partially supported by Plan Nacional de I+D+I 2013-2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Spanish Ministry of Economy and Competitiveness, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0002 and 0011) - co-financed by the European Development Regional Fund (EDRF) "A way to achieve Europe". M.F.R. holds a clinical research contract "Juan Rodés" (JR14/00036) from the Instituto de Salud Carlos III, Spanish Ministry of Economy and Competitiveness.
- **Corresponding author:** Mark Reinwald, MD. Department of Hematology and Oncology, Klinikum Brandenburg, Hochstraße 29, Brandenburg an der Havel 14770, Germany. Email address: [mark.reinwald@medma.uni-heidelberg.de](mailto:mark.reinwald@medma.uni-heidelberg.de)

**Abstract** (250 words)

*Background:* The present review is part of the ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies.

*Aims:* To review, from an Infectious Diseases perspective, the safety profile of therapies targeting different intracellular signaling pathways and to suggest preventive recommendations.

*Sources:* Computer-based MEDLINE searches with MeSH terms pertaining to each agent or therapeutic family.

*Content:* Although BCR-ABL tyrosine kinase inhibitors modestly increase the overall risk of infection, dasatinib has been associated to cytomegalovirus (CMV) and hepatitis B virus (HBV) reactivation. BRAF/MEK kinase inhibitors do not significantly impact infection susceptibility. The effect of Bruton's tyrosine kinase inhibitors (ibrutinib) among patients with B-cell malignancies is difficult to distinguish from that of previous immunosuppression. However, cases of *Pneumocystis pneumonia* (PCP), invasive fungal infection and progressive multifocal leukoencephalopathy have been occasionally reported. Since phosphatidylinositol-3-kinase inhibitors (idelalisib) may predispose to opportunistic infections, anti-*Pneumocystis* prophylaxis and prevention strategies for CMV are recommended. No increased rates of infection have been observed with venetoclax (antiapoptotic protein Bcl-2 inhibitor). Therapy with Janus kinase inhibitors markedly increases the incidence of infection. Pre-treatment screening for chronic HBV and latent tuberculosis infection must be performed, and anti-*Pneumocystis* prophylaxis should be considered for patients with additional risk factors. Cancer patients receiving mTOR inhibitors face an increased incidence of overall infection, especially those with additional risk factors (prior therapies or delayed wound healing).

*Implications:* Specific preventive approaches are warranted in view of the increased risk of infection associated to some of the reviewed agents.

**Keywords:** BCR/ABL kinase inhibitors; small-molecule inhibitors; ibrutinib; idelalisib; Janus kinase inhibitors; mTOR inhibitors; infection.

## Introduction

The present review paper is part of a larger effort launched by the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) Study Group for Infections in Compromised Hosts (ESGICH) and aimed at analyzing, from an Infectious Diseases perspective, the safety profile of biological and targeted therapies. By means of a set of unrestricted computer-based MEDLINE (National Library of Medicine, Bethesda, MD) searches based on the MeSH terms appropriate for each agent or therapeutic family, we identified literature pertaining to the subject. In addition, package information and boxed warning alerts from regulatory agencies (European Medicines Agency [EMA] and US Food and Drug Administration [FDA]) were reviewed. Methodological details are provided in the Introduction section of the present Supplement issue [1]. For each agent (or class of agents), a common outline is offered: (a) summary of mechanism of action, approved indications and most common off-label uses; (b) theoretically expected impact on the host's susceptibility to infection; (c) available evidence emerging from the clinical use of that agent (i.e., randomized clinical trials [RCTs], post-marketing studies, case series and single case reports); and (d) suggested preventive and risk minimization strategies. This section is devoted to review the risk of infection entailed by the use of anti-neoplastic agents targeting tyrosine kinases and other key signaling proteins. It should be noted that the impact of antiangiogenic agents (such as monoclonal antibodies against the vascular endothelial growth factor [VEGF] and its receptor or VEGF tyrosine kinase inhibitors), antibodies against the epidermal growth factor receptor, and inhibitors of the intracellular tyrosine kinase domain of cell surface receptors of the ErbB family (including the so-called multi-kinase inhibitors) has been covered in the preceding section of the document.

**Table 1** summarizes the development status, approved indications and theoretical impact on infectious susceptibility of the reviewed agents, whereas the suggested strategies to prevent such complications are depicted in **Table 2**. It should be emphasized, however, that in view of the limited data available so far for many of these agents, the provided recommendations are necessarily open for constant modifications on the basis of ongoing and future clinical observations. An increased awareness by clinicians is required to identify emerging infections occurring in patients treated with tyrosine kinase inhibitors, to report them promptly and to

collect information systematically within multicenter collaborative groups in order not to miss uncommon but relevant events.

### **BCR-ABL tyrosine kinase inhibitors: imatinib, dasatinib, nilotinib, bosutinib and ponatinib**

#### *Mechanism of action, approved indications and off-label uses*

Chronic myeloid leukemia (CML) is characterized by the (9;22)(q34;q11) translocation (cytogenetically visible as the Philadelphia chromosome [Ph]), which gives rise to the breakpoint cluster region gene-Abelson murine leukemia viral oncogene homolog 1 (BCR-ABL) fusion protein, a constitutively active tyrosine kinase (TK) that induces cell survival and proliferation. Imatinib (Glivec<sup>®</sup> or Gleevec<sup>®</sup>, Novartis Pharmaceuticals) was approved in 2001 as the first TK inhibitor for the treatment of Ph+ CML. Imatinib binds to the ATP-binding pocket of the BCR-ABL protein, thus preventing the kinase to become active. This agent also blocks other TKs, such as the KIT (c-Kit) receptor, the stem cell factor (SCF) receptor, the discoidin domain receptors (DDR1 and DDR2) or the platelet-derived growth factor (PDGF) receptors (PDGFR- $\alpha$  and - $\beta$ ) [2,3]. Imatinib is currently approved as first-line therapy for newly diagnosed Ph+ CML in adults and children who are not suitable for hematopoietic stem cell transplantation (HSCT), or for those in blast, accelerated or chronic phases after failure of interferon- $\alpha$  (IFN- $\alpha$ ) therapy. In addition, it is approved for relapsed or refractory Ph+ acute lymphoblastic leukemia (ALL), myelodysplastic or myeloproliferative diseases associated with *PDGFR* gene rearrangements, aggressive systemic mastocytosis without the D816V c-Kit mutation or with mutational status unknown, hypereosinophilic syndrome and/or chronic eosinophilic leukemia, and unresectable, recurrent and/or metastatic dermatofibrosarcoma protuberans. Imatinib is the only first-line targeted therapy approved for patients with c-Kit-positive gastrointestinal stromal tumors (GIST), both as adjuvant therapy following resection and in the advanced/metastatic setting [2,3].

Dasatinib (Sprycel<sup>®</sup>, Bristol-Myers Squibb) is a second-generation multitargeted TK inhibitors that binds to the active and inactive forms of the BCR-ABL kinase (as opposed to imatinib, which only binds to the inactive state). It has been shown in vitro to exert a 300-fold more potent inhibition than imatinib, being effective against most imatinib-resistant BCR-ABL mutations. Dasatinib also targets the SRC family kinases, c-Kit, PDGFR- $\alpha$  and - $\beta$ , DDR1 and ephrin receptors. This TK inhibitor is currently approved for newly diagnosed Ph+ CML in chronic

phase, as well as for patients with Ph+ CML in any phase or Ph+ ALL and resistance or intolerance to prior therapy, including imatinib [2,3].

Nilotinib (Tasigna<sup>®</sup>, Novartis Pharmaceuticals) was 20 to 30 times more potent than imatinib in preclinical studies. Nilotinib inhibits most imatinib-resistant BCR-ABL mutations, as well as c-Kit, PDGFR, DDR1, VEGF and ephrin receptors. It is recommended as first-line treatment of newly diagnosed Ph+ CML in chronic phase and for patients with chronic or accelerated phases that were resistant to or intolerant to prior therapy, including imatinib [2,3].

Bosutinib (Bosulif<sup>®</sup>, Pfizer) is other dual-specific inhibitor of the SRC and ABL kinase families that remains active against most BCR-ABL resistance mutations, although it has minimal activity against PDGFR and c-Kit. More potent than imatinib, bosutinib has been approved for CML in patients that have developed resistance or intolerance to previous therapies [2].

Ponatinib (Iclusig<sup>®</sup>, Incyte Corporation) is a third-generation multitargeted TK inhibitor that exhibits a unique carbon-carbon triple bond allowing BCR-ABL kinase inhibition even in presence of the T315I mutation, which alters the topology of the ATP-binding region [2]. It is approved for patients with Ph+ ALL or CML (in all phases of disease) that were resistant or intolerant to prior TK inhibitor-based therapies.

#### *Expected impact on the infection risk*

Myelotoxicity is one of the most important adverse effects associated with TK inhibitors, particularly among patients with advanced disease (i.e., blastic phase CML) [4]. Resulting neutropenia is expected to increase the risk of bacterial infection. It has been demonstrated that TK inhibitors also inhibit CD4+ and CD8+ T-cell proliferation in a dose-dependent manner through an off-target kinase inhibition. By inhibiting LCK, a member of the SRC family of TKs that phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) on the T-cell receptor, imatinib interferes with T-cell activation and impairs cytomegalovirus (CMV)- and Epstein-Barr virus (EBV)-specific CD8+ T-cell responses [5]. *In vitro* studies have shown that imatinib also inhibits the differentiation and function of CD34+ dendritic cells (DCs) [6] and CD4+CD25+ regulatory T-cells [7]. Both nilotinib and dasatinib have been associated with an inhibition of CD8+ T-cell proliferation [8,9]. Similar to imatinib, dasatinib inhibits the proliferation of CMV-specific CD8+ T-cells, as well as influenza matrix protein (IMP)-specific CD8+ T-cell responses [9]. A recent study comparing the cellular and humoral responses to influenza and

pneumococcal vaccines in CML patients on imatinib, dasatinib or nilotinib reported that TK inhibitors significantly impair B-cell responses. TK inhibitor-treated patients had significantly lower anti-pneumococcal IgM titers and lower frequencies of peripheral blood IgM memory B-cells after vaccination compared with healthy controls [10].

#### *Available clinical data*

An initial trial that compared imatinib versus IFN- $\alpha$  plus low-dose cytarabine for CML showed only a moderate increase in the incidence of upper respiratory tract infection in the former group [11]. A subsequent RCT involving 250 imatinib-treated patients concluded that bacterial infection had a minor clinical impact and that the occurrence of opportunistic infection was unusual [12]. Phase 2 trials evaluating imatinib and conventional chemotherapy for Ph+ ALL did not find significant differences in the risk of febrile episodes or documented infections compared with that observed with conventional chemotherapy alone [13,14]. Nevertheless, reactivation of hepatitis B virus (HBV) infection under imatinib treatment for CML has been repeatedly reported [15-18]. HBV reactivation was also described in two patients receiving imatinib for non-hematological conditions [19,20]. Some of these cases presented as fulminant hepatic failure, and at least 2 of them were successfully treated with liver transplantation and antiviral medication [17,19]. Although the mechanism underlying this complication is not fully completely understood, it appears to be associated with the inhibition of the T-cell response (which would allow intensive HBV replication) and the subsequently immune reconstitution (which would trigger the immune-mediated injury of infected hepatocytes) [17]. A retrospective analysis of 771 CML patients treated with imatinib found a rate of about 2.0% for varicella-zoster virus (VZV) *de novo* infection or reactivation (5.25 cases per 100 patient-years) [21]. Of note, VZV infection was associated with a longer course of CML and a more intensive prior therapy, was not associated with disseminated forms, and responded well to antiviral therapy. A case of herpes zoster (HZ) complicating imatinib therapy in a patient with GIST has been also reported [22], as well as anecdotal examples of disseminated EBV infection [23], pulmonary [24,25] and peritoneal tuberculosis [26], and nocardiosis [27].

Data on nilotinib-related infection is scarce. Initial trials comparing nilotinib with imatinib did not describe in detail the occurrence of infectious complications [28,29]. A retrospective multicenter analysis on 88 CML patients treated with nilotinib found that 7 of them (7.9%) developed



infection (including one case of perianal mycosis) [30]. A nilotinib-related HBV reactivation has been recently reported [18].

A safety analysis of pooled data from dasatinib trials concluded that serious infections were rare, with only one case of grade 3-4 opportunistic infection [31]. However, a retrospective analysis of 69 dasatinib-treated patients reported an incidence of infection of 51% (being pneumonia and soft-tissue infections the most common forms), with two cases of infection-attributable death. Only one episode of proven invasive fungal infection (catheter-related *Candida krusei* bloodstream infection) was observed. Patients with infection were significantly more likely to have ALL and to have received high-dose corticosteroids. In the multivariate analysis, treatment with three or more cycles of dasatinib increased the risk of infection [32]. A phase 3 study comparing two dosing regimens of dasatinib for Ph+ ALL (140 mg once daily versus 70 mg twice daily) reported an incidence of infection of 18% (8% for grade 3-4 events) in the once-daily arm [33]. A recent study found that the use of dasatinib in HSCT recipients to prevent or preemptively treat molecular relapse of Ph+ hematological malignancies significantly increased the risk of CMV reactivation during the first post-transplant year (adjusted hazard ratio of 7.65 after controlling for acute graft-versus-host disease) [34]. Further cases of CMV disease (hepatitis and colitis) associated with the use of dasatinib have been reported [35-38]. Finally, there have been sporadic reports of *Pneumocystis jiroveci* pneumonia (PCP) [39,40], HBV reaction [41], parvovirus B19 infection and human herpesvirus 6 reactivation [42].

Data on the risk of infection with bosutinib and ponatinib is still scarce. The BELA trial, which compared bosutinib and imatinib for CML, reported similar rates of upper respiratory tract infection (12% and 8%, respectively), with no cases of grade 3-4 infection [43,44]. A phase 2 trial including 449 CML and ALL patients treated with ponatinib reported 6 cases of infection-attributable death (1.3%). Nevertheless, only two of them were deemed by the investigators to be ponatinib-related [45].

Data on the cumulative impact on infection susceptibility resulting from the sequential use of different BCR-ABL tyrosine kinase inhibitors in patients with CML and resistance or intolerance to first- or second-line agents is currently limited [46]. Most studies, usually with low sample sizes, did not specifically report the occurrence of infectious complications [47-49]. Only one episode of infection (in the setting of grade 3-4 neutropenia) was observed in a phase 1/2 trial

with bosutinib in 118 patients previously treated with imatinib followed by dasatinib and/or nilotinib [50]. However, evaluation of cross-intolerance found that 22% of patients with dasatinib intolerance experienced the same adverse event on bosutinib as a grade 3/4 event, suggesting that the development of deeper neutropenia might be expected with second or third lines of therapy.

#### *Conclusions and suggested prevention strategies*

- In view of available data, therapy with BCR-ABL TK inhibitors implies a modest increase in the risk of infection, most likely due to off-target inhibition of kinases involved in immune cells functionality rather than direct inhibition of the BCR-ABL signaling pathway.
- Dasatinib treatment appears to be associated with a higher rate of infectious complications, particularly CMV infection and HBV reactivation among HSCT recipients.
- Screening for chronic HBV infection should be performed before starting treatment with BCR-ABL TK inhibitors. Antiviral prophylaxis while on therapy should be offered to hepatitis B surface antigen (HBsAg)-positive patients for preventing HBV reactivation. In addition, monitoring for HBV viral load among hepatitis B core antibody (anti-HBc)-positive, HBsAg-negative patients could be indicated to assess the eventual reactivation of occult HBV infection. Alternatively, hepatitis specialist referral could be considered.
- No benefit is expected from the universal use of antibacterial or antiviral prophylaxis for patients receiving BCR-ABL TK inhibitors, although an individualized infection risk assessment seems advisable. Anti-*Pneumocystis* prophylaxis should be administered according to the general recommendations contained in the current guidelines for non-human immunodeficiency virus (HIV)-infected patients with hematological conditions [51].

#### **BRAF and MEK kinase inhibitors: vemurafenib, dabrafenib, trametinib, cobimetinib, selumetinib and encorafenib**

##### *Mechanism of action, approved indications and off-label uses*

In the mitogen-activated protein kinase (MAPK) activating pathway, Ras oncoproteins activate Raf, MEK and ERK kinases to direct key cell proliferative and survival signals (**Figure 1**). Activating mutations of the B-type Raf kinase (*BRAF*) oncogene are present in approximately 5-10% of all human malignancies and lead to constitutive activation of the MAPK pathway. In

detail, nearly half of the patients with advanced melanoma harbor the V600E mutation in the *BRAF* gene (other less common mutations include V600K or V600R). Since the FDA approval of BRAF inhibitors for metastatic melanoma in 2011, and the subsequent introduction of combination therapy with MEK inhibitors, the outcome of patients with metastatic melanoma has dramatically changed. Survival has been increased from months to years, with long-term control in a minority of patients [52]. Four compounds have been FDA-approved as mono- or combination therapy for metastatic *BRAF* V600E/K-mutant melanoma. Vemurafenib (Zelboraf<sup>®</sup>, Roche) and dabrafenib (Tafinlar<sup>®</sup>, GlaxoSmithKline) are BRAF inhibitors, whereas trametinib (Mekinist<sup>®</sup>, Novartis Pharmaceuticals) and cobimetinib (Cotellic<sup>®</sup>, Roche) are MEK1/2 inhibitors [52]. The ultimate mode of action of these agents is not entirely understood but appears to involve stimulation of T-cell proliferation and enhanced immune recognition of melanoma. In addition, the combination of dabrafenib and trametinib has been recently granted for non-small cell lung carcinoma harboring *BRAF* V600 mutations. Selumetinib and encorafenib are still in early phases of development.

#### *Expected impact on the susceptibility to infection*

While some of the antitumor effect of BRAF and MEK kinases inhibition is believed to be mediated via the immune response (e.g. natural killer [NK] cells), targeting these pathways does not result in any apparent immunosuppression. Therefore, infection susceptibility is not expected to be directly increased.

In fact, the contrary may be the case. The MEK signaling pathway is involved in influenza virus replication, and combination therapy with oseltamivir and MEK inhibitors showed *in vitro* synergistic activity [53]. The MEK pathway is also involved in the regulation of FoxP3, a crucial transcription factor that controls function and suppressive activity of regulatory T-cells (Tregs). *Ex vivo* MEK inhibition with trametinib in blood samples obtained from HIV-infected patients with tuberculosis downregulated resting and activated Tregs and reduced the production of pro-inflammatory cytokines in stimulated T-cells, resulting in a net improvement of host's immune response by decreasing the chronic pro-inflammatory state [54]. Trametinib suppresses lipopolysaccharide-induced tumour necrosis factor (TNF)- $\alpha$  production and endotoxin shock [55]. Further studies suggest that trametinib may block Merkel cell human polyomavirus (HPyV) infection in fibroblasts [56] or exert some antischistosomal activity [57]. Taken together, these

findings support a potential antimicrobial effect. Moreover, this research line opens interesting prospects for the eventual antiviral activity exerted by these agents and their added value in certain neoplasms in whose pathogenesis oncogene viruses play an active role.

All available data hitherto are in line with the assumption that BRAF and MEK kinases inhibition has no immunosuppressive effects.

#### *Available clinical data*

The most common adverse effects in the landmark study comparing vemurafenib with dacarbazine for metastatic melanoma were arthralgia, rash, fatigue, nausea, diarrhea and cutaneous squamous-cell carcinoma or keratoacanthoma [58]. Some of these events may mimic an infectious etiology, but were attributed to a direct effect by the drug in almost all cases. The high incidence of squamous-cell carcinoma or keratoacanthoma, some of which are mediated by human papillomaviruses (HPV) and/or HPyV, prompted additional research to clarify whether BRAF inhibition has a role in viral activation and subsequent development of skin tumors. The limited data available so far has been conflicting, with some studies showing an association [59] while others failing to demonstrate any apparent connection [60]. An extended follow-up of the initial BRIM-3 trial [58] did not reveal any additional safety issues regarding infectious events [61]. A similar lack of association was reported in the comparator trial of cobimetinib with vemurafenib [62]. In a single center study vemurafenib and dabrafenib were compared for the effect on lymphocyte counts. Vemurafenib therapy decreased lymphocyte counts and altered CD4+ T-cell phenotype and function when compared to dabrafenib [63]. In a further analysis the concomitant use of systemic corticosteroids and vemurafenib was found to induce a more profound lymphopenia, which was believed to contribute to the occurrence of infection in some patients (with two of them dying from pneumonia). Unfortunately no further details were given as to the nature of the pneumonia [64]. The occurrence of a sterile scrotal abscess was reported in one patient under vemurafenib therapy, believed to be related to the therapy [65].

Comparable to vemurafenib, dabrafenib therapy did not result in any measurable increase in the risk of infection in large trials. However, the more common adverse effects included skin-related toxic effects, pyrexia and fatigue, again suggestive of an infectious etiology [66]. The most challenging issue may therefore be the distinction between drug-induced toxicities and ongoing

infection. No association between the observed skin toxicity in dabrafenib-treated patients and HPV infection was demonstrated with immunohistochemical examination of skin samples [67]. On the other hand, the successful use of dabrafenib has been reported in a patient with refractory hairy cell leukemia diagnosed in the previous month with invasive pulmonary aspergillosis [68].

In phase 2 and 3 studies with trametinib in combination with dabrafenib the occurrence of fever and chills was one of the most common adverse effects observed, although it was directly attributed to the drug combination. No specific infection risk was found [69-71].

Clinical data is still limited with selumetinib and cobimetinib, but the safety profile is expected to be in line with the one of trametinib. Selumetinib has been tested for recurrent or persistent endometrial cancer but was not pursued further due to the lack of efficacy [72]. Encorafenib is still at a very early stage of development, and no clinical data exist so far.

Finally, the risk of infection was not increased with the use of BRAF and MEK inhibitors in a meta-analysis [73] and two reviews on the management of most commonly observed toxicities [74,75].

#### *Conclusions and suggested prevention strategies*

- In view of available data, therapy with BRAF and MEK kinase inhibitors does not increase the risk of infection. However, a major clinical challenge is the mimicry of an ongoing infectious complication by some of the most common drug-related adverse effects observed with this therapy (i.e., pyrexia, fatigue, arthralgia and rash).
- No specific prevention strategies are recommended for patients receiving BRAF and MEK inhibitors, although continuous clinical surveillance is advisable since the underlying mechanisms of action are still poorly understood and rare infections may have been missed given the limited drug exposure so far.

#### **Bruton's tyrosine kinase (BTK) inhibitors: ibrutinib and acalabrutinib**

##### *Mechanism of action, approved indications and off-label uses*

Ibrutinib (Imbruvica<sup>®</sup>, Janssen) is an inhibitor of the Bruton's TK (BTK), an important signaling molecule of the B-cell receptor (BCR) and cytokine receptor pathways. The BCR pathway is implicated in the pathogenesis of several B-cell malignancies, including chronic lymphocytic

leukemia (CLL), diffuse large B-cell lymphoma, mature (peripheral) B-cell neoplasm small lymphocytic lymphoma (SLL), follicular lymphoma, and mantle cell lymphoma. Preclinical studies have shown that ibrutinib inhibits numerous processes, including ERK kinase signaling, nuclear factor-Kappa B (NF- $\kappa$ B) DNA binding, cytosine-phosphate-guanine (CpG)-mediated CLL-cell proliferation, and tumor-cell migration [76]. However, ibrutinib does not have toxic effects on normal T-cells, which distinguishes it from most conventional regimens used for CLL [76]. Ibrutinib as a single agent is indicated for the treatment of adult patients with previously untreated CLL or, in combination with bendamustine and rituximab, for those that have received at least one prior therapy. In addition, ibrutinib as a single agent has been approved for relapsed or refractory mantle cell lymphoma and Waldenstroem's macroglobulinaemia in patients who have received at least one prior therapy or as first-line treatment for those deemed unsuitable for chemo-immunotherapy. It was also recently granted an accelerated FDA approval for the treatment of marginal zone lymphoma.

Acalabrutinib (ACP-196, Acerta Pharma BV) is a second-generation, more selective, irreversible BTK inhibitor with improved pharmacologic features, including a more favorable plasma exposure, rapid oral absorption, short half-life, and the absence of irreversible targeting to alternative kinases. As compared to ibrutinib, which also targets ERK and other kinases, acalabrutinib exerts a more selective action on BTK [74]. Acalabrutinib has obtained Breakthrough Therapy Designation from the FDA for the treatment of patients with mantle cell lymphoma who have received at least one prior therapy.

#### *Expected impact on the infection risk*

Mutation of the *BTK* gene causes X-linked (or Bruton's) agammaglobulinemia. Patients suffering from this primary immunodeficiency exhibit a block in early B-cell maturation that prevents development of antibody-producing cells, with the subsequent phenotype consisting of severe, life-threatening bacterial infections [77]. Therefore, an impairment of humoral immunity eventually leading to the development of hypogammaglobulinemia (HGG) could be *a priori* expected among patients treated with BTK inhibitors. On the contrary, some studies have reported an increase in peripheral blood B-cell counts during the course of treatment with ibrutinib, as well as a more rapid immune reconstitution and a significantly lower rate of infection as compared with conventional chemotherapy. These findings would suggest in fact that

ibrutinib allows for a clinically meaningful recovery of humoral immune function in patients with CLL and other B-cell malignancies [78].

*Available clinical data*

The benefit of ibrutinib for relapsed or refractory CLL have been demonstrated in several prospective clinical trials [79-81]. In a phase 1b-2 study to assess the safety and efficacy of ibrutinib in patients with relapsed or refractory CLL or SLL [80], long-term therapy with this agent was associated with modest toxicity, since most adverse events were grade 1 or 2. Pneumonia (occurring in 10 patients [12%]) was the most common adverse event of grade 3 or higher. The average rate of infection was 7.1 per 100 patient-months throughout the first 6 months and 2.6 per 100 patient-months thereafter. In addition, ibrutinib caused a transient increase in peripheral blood total lymphocyte counts [80]. In a phase 3 study comparing ibrutinib versus ofatumumab (a CD20-targeted monoclonal antibody) for relapsed or refractory CLL, the rate of adverse events of grade 3 or higher (including diarrhea and new-onset atrial fibrillation) was increased in the ibrutinib group. Infections of any grade were also more common with ibrutinib (70% versus 54%), although the occurrence of episodes of grade 3 or higher was similar across both groups (24% versus 22%). Following upper respiratory tract infections, pneumonia and urinary tract infection (with rates of about 10% among ibrutinib-treated patients) were the most commonly observed syndromes [79]. In a phase 3 trial of ibrutinib (versus placebo) in combination with bendamustine and rituximab for previously treated CLL or SLL, a safety profile similar to that previously reported for each treatment arm individually was noted (including the occurrence of neutropenia in more than 50% of patients) [81]. The overall proportion of patients with any adverse event or grade 3-4 adverse events did not significantly differ across groups. In detail, infection of any grade (70% in both groups) and of grade 3 or higher (29% in the ibrutinib group and 25% in the placebo group) occurred similarly. A higher incidence of atrial fibrillation was reported again in patients treated with ibrutinib [81]. Finally, the safety of ibrutinib in a phase 3 trial for previously untreated older patients with CLL or SLL (who often had clinically significant comorbidities) was consistent with that observed in previous reports. Serious pneumonia and upper respiratory tract infection occurred in 4% and 2% of patients, respectively [82].

The experience with ibrutinib for malignancies other than CLL or SLL is also relevant [83-85].

Pneumonia of grade 3 or higher was the most common infection among patients with relapsed or refractory mantle cell lymphoma and other types of non-Hodgkin's lymphoma treated with ibrutinib (with a rate of approximately 6%) [83]. The safety profile was favorable for ibrutinib as compared to temsirolimus in patients with relapsed or refractory mantle cell lymphoma [84]. In a study for previously treated Waldenstroem's macroglobulinemia, one ibrutinib-treated patient with IgA and IgG HGG developed streptococcal bacteremia and infective endocarditis after a dental procedure [85]. However, the occurrence of infections deemed to be ibrutinib-related was uncommon since most patients that developed infection had preexisting HGG [85].

Although the expected impact on T-cell function is low, opportunistic infections have been sporadically reported in patients treated with ibrutinib, including cryptococcosis [86-88], PCP [89,90], histoplasmosis [91], invasive aspergillosis [92,93] and disseminated fusariosis [94]. Of note, cases of fatal progressive multifocal leukoencephalopathy (PML) have been reported following the use of ibrutinib in the context of multiple prior treatment lines, including rituximab [95,96]. A recent literature review on the occurrence of invasive fungal infections, including PCP, cryptococcosis and invasive mold infection, among patients treated with ibrutinib has drawn attention on the plausibility that this agent could exert a deleterious off-target effect on the T cell-macrophage axis. Moreover, the authors emphasized the interplay between disease-related factors (i.e., status of underlying malignancy or comorbid conditions), environmental exposures to fungal conidia, and synergy with other immunosuppressive therapies, in conferring an increased susceptibility to fungal pathogens among patients treated with ibrutinib and other tyrosine kinase inhibitors [97]. Since relapsed CLL patients often harbor additional risk factors for PCP (such as multiple purine analog-based regimens or high-dose corticosteroids) [51], some experts advocate for the administration of anti-*Pneumocystis* prophylaxis throughout the entire course of ibrutinib therapy [98].

Safety data for acalabrutinib are still limited. In an uncontrolled phase 1-2 trial including 61 patients with relapsed CLL most observed adverse events were of grade 1 or 2. Upper respiratory tract infection occurred in 23% of patients, and only one death (due to pneumonia) was observed during the course of the trial [99]. A phase 3 study (ClinicalTrials.gov number NCT02477696) has commenced in which acalabrutinib is being compared with ibrutinib for high-risk patients with relapsed CLL.



*Conclusions and suggested prevention strategies*

- In view of available data, therapy with BTK inhibitors modestly increases the risk of infection. However, it is difficult to discern the attributable risk since these agents are usually used in combination with other immunosuppressive drugs in previously treated patients with B-cell malignancies that may be associated with inherent immune defects.
- The occurrence of infection (including pneumonia, PCP and invasive fungal infection) has been observed in ibrutinib-treated patients, especially in presence of neutropenia.
- Although no benefit is expected from the universal use of antibacterial or antifungal prophylaxis, patients receiving ibrutinib should be closely monitored for fever or neutropenia, and appropriate anti-infective therapy should be instituted if appropriate.
- Anti-*Pneumocystis* prophylaxis should be administered according to the current guidelines for non-HIV-infected patients with hematological conditions [51], especially in those with relapsed or refractory CLL and additional risk factors for PCP (i.e., alemtuzumab, purine analogue-based chemotherapy or prolonged high-dose corticosteroids).
- PML is a life-threatening complication occasionally associated with the use of ibrutinib. The new onset of neurological symptoms in ibrutinib-treated patients should prompt clinical suspicion and early treatment discontinuation, followed by appropriate diagnostic work-up.

**Phosphatidylinositol-3-kinase (PI3K) inhibitors: idelalisib, buparlisib, rigosertib and duvelisib***Mechanism of action, approved indications and off-label uses*

The Ras/phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway constitutes a critical signaling pathway frequently altered in human cancer. The PI3K is a lipid kinase that transmits signals from different surface receptors, such as BCR, thereby regulating cellular growth, survival and migration [100]. It is comprised of a p85 regulatory and a p110 catalytic subunit with four different isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ). The PI3K $\delta$  signaling pathways are frequently overexpressed in B-cell malignancies, thus making its inhibition a promising therapeutic approach for CLL and SLL [101]. Idelalisib (Zydelig<sup>®</sup>, Gilead) is a potent small-molecule PI3K inhibitor with highly selective activity against the  $\delta$  isoform [102]. Idelalisib is currently indicated in combination with a CD20-targeted monoclonal antibody (rituximab or ofatumumab) for the treatment of adult patients with CLL after at least one prior therapy or as

first line treatment in the presence of 17p deletion (del[17p]) or *TP53* mutations in patients not eligible for alternative therapeutic approaches. It is also indicated as monotherapy in patients with refractory follicular lymphoma [103]. Buparlisib, rigosertib and duvelisib are other PI3K inhibitors (some of them with additional action on the polo-like kinase 1 [PLK-1] signaling pathway) still at early phases of clinical development.

#### *Expected impact on the infection risk*

*In vitro*, idelalisib significantly reduced chemotaxis toward CXCL12 and CXCL13, disrupted BCR signaling and interrupted paracrine chemokine production by LLC cell lines [104]. In addition to the role displayed by the PI3K/Akt/mTOR pathway in the survival of cancer cells, its importance in the homeostasis of normal, non-tumor cells cannot be overstated. This signaling pathway contributes to regulation of cytokine production by immune cells [105] and, therefore, a risk of infection across all PI3K-targeted drugs has been communicated [106].

#### *Available clinical data*

Data on the safety profile of idelalisib mainly come from various phase 1/2 studies and two larger phase 3 trials [103]. The most frequently reported adverse drug reactions were rash, pyrexia, diarrhea, neutropenia, pneumonitis, hepatotoxicity and infection. Serious infections described with idelalisib therapy include PCP and CMV disease [103]. No infectious agents have been documented in patients with idelalisib-related diarrhea, and an autoreactive T-cell-mediated mechanism has been postulated [103]. The occurrence of fatal or serious pneumonitis not responding to conventional antimicrobial therapy has been reported in about 4% of idelalisib-treated patients (37/895) recruited across 4 RCTs in comparison to 1% (6/548) in the comparator arm [103].

In a phase 3 trial that assessed the efficacy and safety of idelalisib plus with rituximab versus rituximab plus placebo in relapsed CLL, idelalisib significantly improved progression-free and overall survival [107]. The most frequent serious adverse events in the idelalisib and placebo groups were pneumonia (6% versus 8%, respectively), pyrexia (6% versus 3%) and febrile neutropenia (5% versus 6%). The rates of PCP among idelalisib-exposed and unexposed patients were 3% and 1% [107]. In a single-group, phase 2 study for indolent non-Hodgkin's lymphomas that had not responded to rituximab and an alkylating agent or had had early relapse, the most commonly observed adverse events of grade 3 or higher were neutropenia

(27%), elevation of aminotransferase levels (13%), diarrhea/colitis (13%) and pneumonia (7%) [108]. Overall, this regimen exhibited a favorable toxicity profile, with low rates of drug discontinuation due to adverse effects.

It is more difficult to delineate the contributing role of idelalisib to the occurrence of infection among CLL patients deemed unsuitable for standard chemotherapy and frequently excluded from clinical trials due to the presence of coexisting illnesses or relapsed markers. The most frequently observed infections are respiratory and septic events. In many instances, the causative agents were not documented, but both *P. jiroveci* and CMV seem to be frequently involved. Nearly all episodes of PCP, including fatal ones, occurred in the absence of specific prophylaxis. A retrospective analysis including data from 8 clinical studies on CLL and SLL evaluated the clinical impact of PCP in these population [109]. Overall, PCP occurred in 2.5% (35/1,391) of idelalisib-treated patients and 0.25% (2/807) of control patients (mostly treated with CD20-targeted and alkylating agents). Of note, only 1.2% (7/598) of patients receiving anti-*Pneumocystis* prophylaxis developed this complication, as compared to 3.5% (28/793) of those without prophylaxis [109]. A *post hoc* analysis of peripheral blood lymphocyte counts among idelalisib-treated patients with PCP or CMV infection suggested that quantitative monitoring may not be useful to properly assess the risk of these opportunistic infections and that the functional dysregulation of immune cells function may predispose to such even in the absence of significant lymphopenia [110]. Finally, an open-label trial comparing idelalisib with ofatumumab versus ofatumumab alone in 261 patients with relapsed CLL confirmed a higher incidence of severe infection in the idelalisib group: pneumonia (13% versus 10%), sepsis (6% versus 1%) and PCP (5% versus 1%) [111]. Currently, the EMA recommends that anti-*Pneumocystis* prophylaxis should be administered to all patients throughout idelalisib therapy and for a period of 2-6 month following discontinuation, and that CMV infection should be regularly monitored among CMV-seropositive patients.

In March 2016 the EMA jointly analyzed the results obtained from 3 RCTs of idelalisib (with or without bendamustine and rituximab) for previously untreated CLL or SLL. An increased risk of death and higher incidence of serious adverse events (including serious and/or fatal hepatotoxicity, colitis and pneumonitis) were noted among subjects receiving idelalisib compared to the control groups. For the EMA these results indicate that idelalisib-related toxicity

is not outweighed by the expected benefits, in view of the favorable prognosis and low disease-related mortality of previously untreated CLL patients. On the basis of these results, this regulatory agency modified the prior terms of the marketing authorizations for idelalisib and considered that such agent should only be used in treatment-*naïve* CLL patients with del(17p) if they are not considered eligible for other therapies. In addition, the FDA has required a specific warning about the risk of fatal and serious idelalisib-related toxicities.

#### *Conclusions and suggested prevention strategies*

- In view of available data, therapy with idelalisib is associated with an increased risk of opportunistic infections (including PCP and CMV infection) and serious and occasionally fatal adverse events (hepatotoxicity, colitis and pneumonitis).
- Anti-*Pneumocystis* prophylaxis is recommended for patients receiving idelalisib throughout the entire course of therapy and for 2-6 month after its discontinuation.
- Regular monitoring for CMV infection during the course of idelalisib therapy is advisable among CMV-seropositive patients or in presence of clinically suspected CMV disease.
- Idelalisib therapy must be discontinued upon the occurrence of suspected pneumonitis, grade 3-4 aminotransferase elevation (>5 times the upper reference limit), or grade 3-4 diarrhoea/colitis.

#### **Antiapoptotic protein Bcl-2 inhibitors: venetoclax**

##### *Mechanism of action, approved indications and off-label uses*

The constitutively elevated expression of the antiapoptotic protein B-cell lymphoma 2 (Bcl-2), encoded by the *BCL2* gene, renders CLL cells resistant to apoptosis, resulting in the accumulation of long-lived, clonal lymphocytes that characterize the disease. Venetoclax (Venclyxto<sup>®</sup>, AbbVie) is a highly selective inhibitor of Bcl-2. *In vitro*, venetoclax induced apoptosis of primary CLL cells and tumor cells that overexpressed *BCL2*, with minimal effects on platelets [112]. Del(17p) is a cytogenetic abnormality leading to the loss of the *TP53* tumor suppressor gene that is found in 3-10% of treatment-*naïve* CLL patients and in up to 50% of those with relapsed or refractory disease. Del(17p) is the most important poor prognosis marker in the context of standard chemo-immunotherapy, as its presence is associated with lower treatment response rate and shorter progression-free and overall survival [113]. There are few

effective therapeutic options for patients with del(17p) CLL. Allogeneic HSCT is potentially curative but only suitable for selected patients. As previously mentioned, ibrutinib monotherapy and idelalisib with rituximab are effective treatments for of greater duration than chemo-immunotherapy in these patients. Venetoclax has been recently approved by the EMA and FDA (under accelerated procedure) for patients with del(17p) CLL who have received at least one prior therapy.

#### *Expected impact on the infection risk*

In addition to its role as regulators of apoptosis, the Bcl-2 family of proteins also has other functions in non-tumor cells, including autophagy, calcium handling, mitochondrial dynamics and energetics) [114]. In a murine model of systemic erythematous lupus, Bcl-2 antagonists selectively killed plasmacytoid DCs (which act as antigen presenting cells) and reduced IFN- $\alpha$  production [115].

#### *Available clinical data*

In a phase 1 study of oral venetoclax in a dose-escalation cohort (from 150 mg to 1,200 mg daily) and an expansion cohort (400 mg daily) for relapsed or refractory CLL or SLL the most important adverse event was tumor lysis syndrome (occurring in 5.4% of patients in the former group) [116]. A relevant feature in this study was the occurrence of neutropenia (considered as grade 3 or 4 in 41% of participants) and febrile neutropenia (in about 6%). Other serious adverse events included pneumonia (4%), upper respiratory tract infection (3%) and immune thrombocytopenia (3%) [116]. A phase 2 single-arm trial assessed the activity and safety of venetoclax monotherapy in 107 patients with relapsed or refractory del(17p) CLL [117]. Of note, the use of antimicrobial prophylaxis was not mandatory. The majority of venetoclax-treated patients experienced a reduction in absolute lymphocyte counts. The most common grade 3-4 adverse events were neutropenia (40.2%) and infection (1.6%). Serious infections occurring in two or more patients were pneumonia (5.6%) and lower or upper respiratory tract infection (1.9% each). One patient died from septic shock and 12 further participants (11.2%) developed infections requiring treatment interruption or dose reduction [117].

In an internal integrated safety analysis of phase 1 and 2 trials evaluating 330 patients with relapsed or refractory CLL that received at least one dose of venetoclax, infections of any grade occurred in approximately 70% of participants [118]. The most common events were upper

respiratory tract infection (23%), pneumonia (11%) and nasopharyngitis (10%). Pneumonia was the predominant grade 3-4 infection and there were 5 cases of infection-attributable death infection (due to septic shock and viral pneumonia). Opportunistic infections occurred in 3.6% of patients and included invasive aspergillosis, PCP, oral and esophageal candidiasis, ocular toxoplasmosis, nocardiosis, herpes pharyngitis and multidermatomal HZ [118]. Venetoclax is a CYP3A substrate and plasma levels are accordingly modified if coadministered with CYP3A inducers or inhibitors [119].

#### *Conclusions and suggested prevention strategies*

- In view of (limited) available data, therapy with venetoclax seems not to be associated with a meaningful increase in the risk of infection, and no benefit is expected from the use of antibacterial, antiviral or anti-*Pneumocystis* prophylaxis.
- Continuous clinical surveillance in patients receiving venetoclax is advisable since the underlying mechanisms are still poorly understood and rare infections may have been missed given the limited drug exposure so far.

#### **Janus kinase (JAK) inhibitors: ruxolitinib, tofacitinib and baricitinib**

##### *Mechanism of action, approved indications and off-label uses*

The family of Janus kinases (JAKs), which comprises four different members (JAK1, JAK2, JAK3 and tyrosine kinase 2 [TyK2]), plays a significant role in haematopoiesis and immune cell signaling and differentiation. JAKs phosphorylate sites on the cytoplasmic tail of a variety of hematopoietic and inflammatory cytokine receptors (i.e., erythropoietin or thrombopoietin receptors), thus effecting downstream targets via the signal transducer and activator of transcription (STAT) pathway. Different JAKs or TyK2 exert differential effects. JAK1 and JAK2 deletions in murine knockout models impair lymphoid and neural development and erythropoiesis, respectively. Lack of TyK2 is associated to a suboptimal interferon response [120]. Loss-of-function mutations in the *JAK3* gene lead to a clinical phenotype of severe combined immunodeficiency [121-124]. As JAK3 is downstream of a variety of cytokine receptors involved in the inflammatory cascade, such as interleukin (IL)-2, IL-4 or IL-21, pharmacological inhibition of these kinases was dubbed to be promising in treating autoimmune diseases or even useful in organ transplantation. In haematological diseases, the V617F

activating mutation in the *JAK2* gene has been identified as one of the major hallmarks in the pathogenesis of myeloproliferative neoplasms (MPN) and has been identified in up to 95% of patients with polycythemia vera (PV) and in 50-60% of patients with myelofibrosis (MF) and essential thrombocythemia [125] [126]. Mutations in JAKs have also been identified in a variety of other haematological malignancies such as acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS) [127].

Currently, there are three EMA-approved JAK inhibitors. Ruxolitinib (Jakavi<sup>®</sup>, Novartis Pharmaceuticals) targets JAK1 and JAK2 and is approved for the treatment of patients with MF [128] or PV (who are resistant to or intolerant of hydroxyurea) [129]. Tofacitinib (Xeljanz<sup>®</sup>, Pfizer [formerly known as tasocitinib]) acts on JAK1, JAK2 and JAK3 (and to a lesser extent on TyK2) and is indicated, in combination with methotrexate, for the treatment of moderate to severe active rheumatoid arthritis (RA) in adult patients who have not responded or are intolerant to disease-modifying antirheumatic drugs (DMARDs) [130]. Baricitinib (Olumiant<sup>®</sup>, Eli Lilly) is a selective and reversible inhibitor of JAK1 and JAK2 that, quite recently, has been demonstrated to be superior than placebo or adalimumab in refractory RA, therefore receiving approval for this condition [131]. In addition, JAK inhibitors are being evaluated for a number of other indications, such as kidney transplantation (KT) [132,133], psoriasis [134], graft-versus-host disease (GvHD) [135], refractory leukemia and solid malignancies [136].

#### *Expected impact on the infection risk*

Preclinical data show a distinct influence of JAK inhibitors on several components of the adaptive immunity. JAK inhibitors impair T-cell function by decreasing the potential of producing proinflammatory cytokines and, therefore, Th1 and Th17 responses result decreased both *in vivo* and *in vitro* [137]. Patients with MPN treated with ruxolitinib showed a profound reduction in Tregs and a silencing of T-cell helper cytokine secretion [138]. Additionally, function and migration of DCs are impeded by ruxolitinib, thus further aggravating immune system dysfunction [139]. Lastly, ruxolitinib exposure led to a severe decrease in NK cell counts, a phenomenon that was linked to an increase in risk of infection [140]. Tofacitinib suppresses cytokine production, proliferation and expansion of CD4<sup>+</sup> T-cells in RA patients [141] and it has been shown to modulate innate and adaptive immune responses by preventing the generation and differentiation of Th1 and Th17 cells [142].

Taken together, immunosuppressive properties of JAK inhibitors are probably mediated by a combination of immune defects. However, other factors (such as previous treatments, concurrent immunosuppressive therapy, pre-existing cytopenias, patient age and comorbidities) most certainly contribute to modulate infection susceptibility.

#### *Available clinical data*

For ruxolitinib, which is the agent with the longest time period since approval, an increased risk of infection has been repeatedly observed in clinical trials. In the pivotal trial that compared ruxolitinib with best available therapy for MF patients, infections were more frequently observed in the experimental arm [143], including urinary tract infection (24.6%), pneumonia (13.1%), HZ (11.5%), sepsis and septic shock (7.9%) and tuberculosis (1.0%) [144]. However, it should be stated that grade 3-4 neutropenia was recognized in 7.1% and 2% of patients in the ruxolitinib and placebo arms, respectively, thus confounding the immunosuppressive potential of ruxolitinib. In addition, long-term follow-up did not show an increase but rather a decrease in the incidence of infection, most likely due to stabilization of the underlying disease [143]. Outside the trial setting, recent real-life data from MF patients treated with ruxolitinib identified several episodes of lethal infection [145], although the long-time evaluation of patients treated within an expanded access program overall reported low incidence and severity of infection [146]. For PV patients, data from a phase 3 trial indicate that ruxolitinib, as compared to standard therapy, was associated with an increased rate of overall (42% versus 37%) and grade 3-4 infections (3.6% versus 2.7%). In particular, HZ was more commonly observed in ruxolitinib-treated patients (6% versus 0%) [129]. In relapsed AML patients treated with ruxolitinib the most common grade 3 or 4 non-hematologic events consisted of infections, especially pneumonia (57.7% [15/26] of patients) [147]. Pneumonia was also much more frequent among patients with metastatic pancreatic carcinoma receiving ruxolitinib compared to placebo (plus capecitabine in both arms) [136]. Safety evaluation of allogeneic HSCT recipients that had been treated with JAK inhibitors prior to transplantation also found atypical and opportunistic infections to occur frequently, including CMV and EBV reactivation (leading to post-transplant lymphoproliferative disorder [PTLD] in one case), BK HPyV-associated hemorrhagic cystitis and invasive fungal infection [148]. In addition, case reports of severe opportunistic infections in patients on ruxolitinib treatment have been repeatedly published, such as HBV reactivation (including occult



HBV infection reactivation in anti-HBc positive, HBsAg-negative patients) [149,150], *Cryptococcus neoformans* pneumonia [151], PCP [152], bilateral *Toxoplasma* chorioretinitis [153], disseminated tuberculosis [154] and PML [155].

As for the use of JAK inhibitors in rheumatologic conditions, most data derives from tofacitinib. In the pivotal RCT an increased rate of serious infections was observed as compared to placebo [156]. A safety analysis of pooled data from RA trials (covering approximately 4,800 patients) found a significant incidence of infection and infection-related mortality with tocilizumab that, however, was similar to that observed with other biological agents. Age, diabetes, prior corticosteroid therapy, low lymphocyte counts and tofacitinib dose were independently linked to the risk of serious infection [157]. A recent meta-analysis evaluating 66 RCTs and 22 long-term extension studies also illustrated a higher incidence of infection with tofacitinib compared with placebo, although such risk was comparable to that observed with ~~associated to~~ other biologic DMARDs [158]. Another recent analysis evaluating the efficacy and safety of tofacitinib in patients with inadequate response to conventional synthetic or biological DMARDs confirmed that patients on concurrent corticosteroid therapy had more serious infections, especially HZ [159].

A recent trial compared tofacitinib (5 or 10 mg daily) with etanercept for the treatment of psoriasis. Rates of infection were similar across study arms, with as the most common events [134]. Two recently published trials in patients with psoriatic arthritis and inadequate response to prior therapy with TNF- $\alpha$ -targeted agents or conventional DMARDs, serious infections (pneumonia and pyelonephritis) and HZ (including cases with multidermatomal involvement) were more common with tofacitinib than placebo [160] or adalimumab [161].

With regards to classic opportunistic infections, Winthrop et al. analyzed phase 1-2 RCTs and long-term extension studies in RA and identified 60 episodes among 5,671 subjects [162]. Tuberculosis was the most common event and was associated with the higher tofacitinib dose (tofacitinib 10 mg twice daily). Importantly, prior treatment of latent tuberculosis infection (LTBI) with isoniazid seemed to be protective. Other opportunistic infections included esophageal candidiasis (9 cases), disseminated or multidermatomal HZ (8 cases), CMV infection (6 cases) and PCP (4 cases) [162]. Based on the immunosuppressive properties of DMARDs including

tofacitinib, screening for viral hepatitis has been proposed for patients on tofacitinib treatment, with prophylaxis for those at medium or high risk of HBV reactivation [163].

Tofacitinib has been also compared to cyclosporine A in a phase 2b trial for KT recipients [132]. Serious infections (including CMV disease and EBV-associated PTLD) were significantly more common among tofacitinib-treated patients. Pharmacokinetic analysis suggested an exposure-dependent mechanism in infection susceptibility [164].

For the newest EMA-approved JAK inhibitor baricitinib, clinical data on the risk of infectious complications can be only extracted from phase 2 and 3 trials. In the initial study comparing different doses with placebo, infection was more frequent among baricitinib-treated patients, although the rate of serious infections was rather comparable across groups (about 3%). Although HZ occurred in all three arms, the largest numbers were seen with baricitinib at the highest analyzed dose (4 mg) [165].

#### *Conclusions and suggested prevention strategies*

- In view of available data, therapy with JAK inhibitors is associated with a markedly increased risk of infection due to the direct suppression of critical components of the immune system.
- Screening for chronic HBV infection should be performed before starting treatment with JAK inhibitors. Antiviral prophylaxis while on therapy should be offered to HBsAg-positive patients for preventing HBV reactivation. In addition, monitoring for HBV viral load among anti-HBc positive, HBsAg-negative patients may be indicated to assess the eventual reactivation of occult HBV infection. Alternatively, specialist referral could be considered.
- Screening for LTBI may also be considered before starting treatment with JAK inhibitors (followed by appropriate therapy if needed), as tuberculosis constitutes the most common opportunistic infection observed.
- Clinicians caring for patients receiving JAK inhibitors should be aware of the increased risk of overall and opportunistic infection (including tuberculosis, PCP, HZ and invasive fungal infection), especially in those with additional risk factors (i.e., prior or concomitant corticosteroid therapy, low lymphocyte counts or high-dose therapy with JAK inhibitors).

- In view of (limited) available data, the administration of antiviral and anti-*Pneumocystis* prophylaxis should be individualized considered, especially in patients with additional risk factors.

### **Mammalian target of rapamycin (mTOR) inhibitors: sirolimus, temsirolimus and everolimus**

#### *Mechanism of action, approved indications and off-label uses*

As previously mentioned, the Ras/PI3K/Akt/mTOR pathway (**Figure 1**) plays a crucial role in cell survival, growth and proliferation [166]. mTOR is a serine/threonine kinase and member of the PI3K-related kinase superfamily [167]. Two distinct mTOR complexes have been identified (mTORC1 and mTORC2). The effects of mTOR on growth, division and metabolism are largely attributable to mTORC1, which is regulated by signals generated from growth factors and cytokines receptors (such as HER2, c-Kit, VEGF and PDGF) and by changes in intracellular ATP content [168]. In addition, it is increasingly clear that many cancer-promoting lesions activate the mTORC1 pathway [169]. The key factor upstream of mTOR is PI3K, which upon activation is able to recruit Akt to the cell membrane that regulates cell metabolism and mTOR activity. PTEN (phosphatase and tensin homolog on chromosome 10) is a phosphatidylinositol-3-phosphatase that negatively regulates this pathway and reverses the action of PI3K [170]. The second mTOR-containing complex (mTORC2) is less understood than mTORC1, but it seems to constitute a critical part of a feedback loop in the PI3K/Akt pathway [171].

The mTORC1 complex regulates protein synthesis through two downstream pathways, namely inactivation of the repressor of mRNA translation 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein) and activation of S6K1 (ribosomal S6 kinase 1) that enhances mRNA translation. By phosphorylating the 4E-BP family of proteins, mTORC1 represses their capacity to inhibit eIF4E (eukaryotic translation initiation factor 4E), thus promoting protein synthesis [172]. Akt is a positive regulator of mTORC1 that phosphorylates and thereby inhibits the heterodimeric tumor suppressor complex (TSC)-1 (harmartin) and TSC2 (tuberin) by removing its inhibitory effect on mTORC1 [168,173]. TSC1/TSC2 inhibits Rheb (Ras homolog enriched in brain), a positive regulator of mTOR that acts downstream of TSC1/TSC2, PI3K and Akt. Aberrant PI3K/mTOR activation is frequently observed in human cancer [174]. The most common underlying mechanism is the loss of *PTEN* gene expression due to deletion or

inactivating mutations. Upregulation can also result from the activation of receptor TKs or alterations of the different isoforms of PI3K [175].

The mTOR inhibitors comprise a unique drug class in possessing both immunosuppressive and anticancer activity. Rapamycin (Rapamune<sup>®</sup>, Pfizer [also known as sirolimus]) and its analogue macrolides everolimus (Certican<sup>®</sup> or Afinitor<sup>®</sup>, Novartis Pharmaceuticals) and temsirolimus (Torisel<sup>®</sup>, Pfizer) act by forming an allosteric inhibitory complex with their intracellular receptor, the immunophilin FK506-binding protein (FKBP12), which binds a region in mTORC1 termed FRB (FKB12-rapamycin binding). Thus, these agents inhibit mTORC1 kinase activity [176] (**Figure 1**). In addition to direct effects on tumor cells, rapamycin also potently inhibits angiogenesis and endothelial cell proliferation [177,178].

The investigation of mTOR inhibitors as anticancer therapies was aided by the fact that rapamycin (sirolimus) had been already approved in 1998 to prevent acute rejection in solid organ transplant (SOT) recipients. Several clinical trials have tested the efficacy of rapamycin and its analogues as anticancer therapy [179]. Analyses of neuroendocrine pancreatic tumors have shown alterations in the mTOR pathway, with downregulation of PTEN and TSC2 observed in most cases [180]. The antineoplastic properties of mTOR inhibitors were first demonstrated for renal angiomyolipoma or pulmonary lymphangiomyomatosis in the setting of tuberous sclerosis complex [181,182] and for Kaposi's sarcoma [183,184]. Experimental and clinical evidence also indicated a role for the PI3K/mTOR pathway in the development of resistance in patients with hormone receptor-positive breast cancer [185].

Everolimus has been approved by the FDA and EMA for the treatment of advanced renal cell carcinoma after failure of VEGFR-targeted therapies (sunitinib or sorafenib) [186-189], advanced neuroendocrine pancreatic tumors [190], advanced hormone receptor-positive HER2-negative breast cancer (in combination with exemestane) [191], and progressive non-functional neuroendocrine gastrointestinal and lung tumors. In addition, everolimus is FDA-approved for subependymal giant cell astrocytoma and angiomyolipoma associated with tuberous sclerosis [192]. Temsirolimus has been approved for advanced renal cell carcinoma. In addition, there are promising results from a phase 3 trial for refractory mantle cell lymphoma [193]. However, even though PTEN loss is frequently observed in sporadic glioma and melanoma, mTOR inhibitors have had only little efficacy in these malignancies [194,195]. In fact, the efficacy of

such agents has also been disappointing in patients with metastatic breast cancer [196,197] despite frequent PI3K activation [174].

#### *Expected impact on infection susceptibility*

In addition to the well-demonstrated direct inhibition on viral replication exerted by mTOR inhibitors (particularly investigated for CMV in the setting of SOT [199-201]), it should be highlighted that mTORC1-mediated functions include both immunosuppressive and immune activating properties. The mTORC1 complex promotes T-cell anergy induces Treg expansion and inhibits maturation of DC [202]. On the other hand, the use of mTOR inhibitors results in the enhancement of central and effector memory CD8<sup>+</sup> T-cell responses after vaccination in nonhuman primates [203]. The role of mTOR in B-cell development and function has recently been reviewed [204]. Relevant to the present review is the notion that patients taking mTOR-inhibitors may have a hampered innate immune response. [205]. The migration of neutrophils to sites of inflammation requires mTOR [206-208], as well as the production of pro-inflammatory cytokines [206,209,210]. The defects in innate immunity may be further compromised by the effect of mTOR inhibition on stromal cells, leading to impaired wound healing [211]. A relevant proportion of mTOR inhibitor-treated patients develop stomatitis and pneumonitis, which may constitute an entry port for pathogenic microorganisms [212]. The mTOR pathway has been also implicated in neutrophil function, including formation of extracellular traps that capture and kill microbes, in a process involving the hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) pathway [213]. About 50-60% of cases of renal cell carcinoma exhibit loss of the Von Hippel-Lindau tumor suppressor, which encodes a negative regulator of HIF1 $\alpha$  [186]. Accordingly, an increased risk of respiratory and genitourinary tract infections with everolimus or temsirolimus has been observed in patients with renal cell carcinoma compared to those with other carcinomas [214].

#### *Available clinical data*

Despite long-term experience with mTOR inhibitors in SOT recipients, the widespread use of these agents has been limited by the relatively high discontinuation rates, reaching up to 20-30% of participants in most transplantation trials [215-219]. These observations posed a significant challenge to the perception of the efficacy of mTOR inhibitors as immunosuppressive and/or antineoplastic agents in relation to their tolerability. The most common adverse effects attributed to mTOR inhibitors include anemia, thrombocytopenia and increased triglyceride

and/or cholesterol levels. Theoretically, therapeutic drug monitoring could be helpful in preventing adverse events [220]. For everolimus, however, a dose-dependent association has only been shown for thrombocytopenia, not for leucopenia or hyperlipidemia [221]. Aphthous stomatitis and diarrhoea are more frequently reported than in patients treated with a calcineurin inhibitors and mycophenolic acid [222-224]. An infrequent but potentially life-threatening adverse effect is non-infectious pneumonitis. This entity is characterized by (nonspecific) inflammatory infiltrates in combination with negative results for infectious causes in blood and bronchoalveolar lavage tests [225]. The incidence of pneumonitis associated with sirolimus or everolimus has been reported between 1 and 12% [226]. No definite risk factors have been identified and, in case of pneumonitis related to a mTOR inhibitor therapy, the drug class should be discontinued.

A systematic review of 12 RCTs reported an increased risk of infectious complications associated with the use of high-dose everolimus or temsirolimus in cancer patients [227]. Dosing strategies of mTOR inhibitors in cancer patients often differ from those used in SOT recipients. Of note, a higher risk of mortality associated to the use of sirolimus has been found in a systemic review based on individual patient data from 21 transplant trials (involving about 6,000 patients). This increased mortality rate was not related to graft loss with return to dialysis, but to cardiovascular and infection-related deaths [228]. A recent meta-analysis utilizing data from 12 phase 2 and 3 trials comparing everolimus or temsirolimus versus placebo in cancer patients also reported a significantly higher risk of infection with mTOR inhibitors, with incidences for all-grade and severe mTOR inhibitor-attributable infection were 9.3% and 2.3%, respectively. The risk substantially varied across different tumor types, being higher for renal cell carcinoma, lymphoma and neuroendocrine tumors. There was no significant difference between everolimus and temsirolimus. Upper respiratory tract infection, urinary tract infection and pneumonia were the predominant forms, with some examples of opportunistic infection (i.e., tuberculosis and HZ) and HBV reactivation. Unfortunately, specific information on the type of infection was not provided in most of included trials [234]. Further case reports have highlighted the risk of HBV reactivation in cancer patients receiving mTOR inhibitors [235,236].

*Conclusions and suggested prevention strategies*

- In view of available data, therapy with mTOR inhibitors in cancer patients is associated with an increased risk of infection, an association that may be partially explained by the different dosing strategies used in this population as compared to SOT recipients.
- Clinicians caring for patients receiving mTOR inhibitors should be aware of the increased risk of overall infection, especially in those with additional risk factors (i.e., certain specific malignancies, prior or concomitant use of potent cancer therapies, or presence of drug-related delay in wound healing or aphthous stomatitis).
- Screening for chronic (latent) infections, including HBV and LTBI, may be advisable before starting treatment with mTOR inhibitors (followed by appropriate prophylaxis or therapy if needed).
- No benefit is expected from the universal use of antibacterial, antiviral or anti-*Pneumocystis* prophylaxis for patients receiving mTOR inhibitors, although it seems advisable to individualize infection risk assessment.

**Figure legends**

**Figure 1.** Key components of Ras/PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways. The mTORC1 and mTORC2 complexes modulate cell cycle via effects on p21, p27, cyclin D1 and E. The mTORC1 complex phosphorylates 4E-BP1 and S6K1 to activate protein translation. Important feedback mechanisms include inactivation of the mTORC2 complex and inhibition of Akt signaling by S6K1-mediated phosphorylation of Rictor and IRS1. Hypoxia, DNA damage and ATP deprivation activate TSC1/TSC2 to restrain mTORC1 and biosynthetic processes in normal tissue. Oncogenic PI3K/PDK1 and Ras/MAPK signaling cooperate to reduce TSC1/TSC2 activity. PTEN, which normally restrains PI3K activity, is also frequently deleted or inactivated in human cancer. mTOR inhibitors disrupt the association between mTOR and Raptor.



**Table 1.** Summary of reviewed inhibitors of Intracellular signaling pathways, mode of action, approved indications and expected impact on immune response.

Agents	Pathway affected	Approved indications (regulatory agency)	Type of regimen	Expected impact of immune function
Imatinib, dasatinib, nilotinib, bosutinib, ponatinib	BCR-ABL, c-Kyt, other off-target kinases	<b>Imatinib:</b> Ph+ CML and ALL, MDS/MPD, hypereosinophilic syndrome and/or chronic eosinophilic leukemia, GIST (FDA and EMA), systemic mastocytosis, dermatofibrosarcoma protuberans (FDA only) <b>Remaining agents:</b> Ph+ CML	Monotherapy or sequential therapy	Neutropenia, reduced T-cell activation and proliferation, inhibition of CD34+ DCs differentiation (imatinib)
Vemurafenib, dabrafenib, encorafenib, trametinib, cobimetinib, selumetinib,	Ras/Raf/ MEK/ERK	<b>All agents:</b> unresectable or metastatic melanoma (FDA and EMA) <b>Dabrafenib, trametinib:</b> NSCLC (EMA only) <b>Selumetinib:</b> thyroid carcinoma (FDA and EMA)	Monotherapy, combination of BRAF and MEK inhibitors	None
Ibrutinib, acalabrutinib	Bruton's tyrosine kinase	<b>Ibrutinib:</b> CLL, WM, mantle cell lymphoma (FDA and EMA), marginal zone lymphoma (FDA only) <b>Acalabrutinib:</b> mantle cell lymphoma (FDA only)	Monotherapy or combined with rituximab and bendamustine (CLL)	Inhibition of BCR signaling and B-cell activation, HGG
Idelalisib, buparlisib, rigosertib, duvelisib	Ras/PI3K/Akt/mTOR	<b>Idelalisib:</b> relapsed/refractory CLL, del(17p) CLL, follicular lymphoma	Monotherapy or combined with rituximab or ofatumumab (CLL)	Inhibition of BCR signaling, reduced chemokine production
Venetoclax	Bcl-2	del(17p) CLL (FDA and EMA)	Monotherapy	Depletion of DCs, reduced IFN- $\alpha$ production (animal model only)
Ruxolitinib, tofacitinib, baricitinib	JAK/STAT	<b>Ruxolitinib:</b> polycythemia vera, myelofibrosis (FDA and EMA) <b>Tofacitinib:</b> rheumatoid arthritis (FDA and EMA) <b>Baricitinib:</b> rheumatoid arthritis (EMA only)	Monotherapy or combined with methotrexate or non-biologic DMARDs (rheumatoid arthritis)	Inhibition of Th1 and Th17 cells differentiation, inhibition of cytokine secretion, reduction of Tregs, impaired DCs function and migration

Sirolimus, everolimus temsirolimus,	Ras/PI3K/Akt/mTOR	<p><b>Sirolimus:</b> SOT (FDA and EMA)</p> <p><b>Everolimus:</b> RCC, breast carcinoma, neuroendocrine tumors (FDA and EMA, tuberous sclerosis-associated tumors (FDA only)</p> <p><b>Temsirolimus:</b> RCC (EMA and FDA), mantle cell lymphoma (EMA only)</p>	Monotherapy or combined with other immunosuppressive agents (SOT)	Impaired innate immunity, reduced neutrophil migration, reduced pro-inflammatory cytokine production
--	-------------------	--	---	--

ALL: acute lymphoblastic leukemia; BCR: B-cell receptor; CCL: chronic lymphocytic leukemia; CML: chronic myeloid leukemia; DC: dendritic cell; del(17p): deletion of 17p; DMARD: disease-modifying antirheumatic drug; EMA: European Medicines Agency; FDA: Food and Drug Administration; GIST: gastrointestinal stromal tumor; HGG: hypogammaglobulinemia; MDS/MPD: myelodysplastic/myeloproliferative disease; NSCLC: non-small cell lung carcinoma; Ph+: positive Philadelphia chromosome; RCC: renal cell carcinoma; SOT: solid organ transplantation; Treg: regulatory T-cell; WM: Waldenstroem's macroglobulinaemia.

**Table 2.** Summary of infection risks and suggested recommendations and management strategies.

Agents	Increased risk of overall infection	Risk of OI	Risk of PCP	Risk of HBV reactivation	Observations and recommendations
Imatinib, dasatinib, nilotinib, bosutinib, ponatinib	Modest	IFI, HZ, tuberculosis, CMV (particularly with dasatinib)	No	Yes	<ul style="list-style-type: none"> <li>• Higher risk of infection with dasatinib (particularly after HSCT)</li> <li>• Screening for chronic HBV infection before starting therapy</li> <li>• Antiviral prophylaxis while on therapy in HBsAg-positive patients</li> <li>• Monitoring for HBV viral load in anti-HBc positive, HBsAg-negative patients to assess eventual reactivation of occult HBV infection</li> <li>• No expected benefit from the universal use of antibacterial, antiviral or anti-<i>Pneumocystis</i> prophylaxis</li> </ul>
Vemurafenib, dabrafenib, encorafenib, trametinib, cobimetinib, selumetinib,	None	No	No	No	<ul style="list-style-type: none"> <li>• No apparent increase in the risk of infection</li> <li>• Some of the most common drug-related adverse effects (pyrexia, fatigue, arthralgia and skin rash) may mimic an ongoing infection</li> </ul>
Ibrutinib, acalabrutinib	Modest	PCP, IFI, PML	Yes (particularly in presence of additional risk factors)	No	<ul style="list-style-type: none"> <li>• Modest increase in the risk of infection (contributing role of prior or concurrent therapies or inherent immune defects)</li> <li>• No expected benefit from the universal use of antibacterial or antifungal prophylaxis</li> <li>• Anti-<i>Pneumocystis</i> prophylaxis for CLL patients with additional risk factors (e.g., purine analogues or high-dose corticosteroids)</li> <li>• PML occasionally associated with the use of ibrutinib</li> </ul>
Idelalisib, buparlisib, rigosertib, duvelisib	Major	IFI, PCP, CMV	Yes	No	<ul style="list-style-type: none"> <li>• Increased risk of OIs and life-threatening adverse events (hepatotoxicity, colitis and pneumonitis).</li> <li>• Anti-<i>Pneumocystis</i> prophylaxis during the course of therapy and for 2-6 month after its discontinuation</li> <li>• Monitoring for CMV infection during the course of therapy in CMV-seropositive patients or in presence of suspected CMV disease</li> <li>• Discontinuation of therapy in presence of suspected pneumonitis or grade 3-4 aminotransferase elevation or diarrhoea/colitis.</li> </ul>

Venetoclax	None	No	No	No	<ul style="list-style-type: none"> <li>• No apparent increase in the risk of infection</li> </ul>
Ruxolitinib, tofacitinib, baricitinib	Major	PCP, HZ, tuberculosis, CMV, EBV, PML	Yes (particularly in presence of additional risk factors)	Yes	<ul style="list-style-type: none"> <li>• Increased risk of overall infection and OIs</li> <li>• Screening for chronic HBV infection before starting therapy</li> <li>• Antiviral prophylaxis while on therapy in HBsAg-positive patients</li> <li>• Monitoring for HBV viral load in anti-HBc positive, HBsAg-negative patients to assess eventual reactivation of occult HBV infection</li> <li>• Screening for LTBI before starting treatment (followed by appropriate therapy if needed)</li> <li>• Anti-<i>Pneumocystis</i> prophylaxis in patients with additional risk factors (e.g., high-dose corticosteroids)</li> </ul>
Sirolimus, everolimus, temsirolimus,	Major	HZ, tuberculosis	No	Yes	<ul style="list-style-type: none"> <li>• Increased risk of infection in cancer patients, especially in those with additional risk factors (i.e., RCC, prior or concomitant cancer therapies, delay in wound healing or aphthous stomatitis).</li> <li>• Screening for chronic HBV infection and LTBI before starting therapy (followed by appropriate therapy if needed)</li> <li>• No expected benefit from the universal use of antibacterial, antiviral or anti-<i>Pneumocystis</i> prophylaxis</li> </ul>

Anti-HBc: hepatitis B core antibody; CCL: chronic lymphocytic leukemia; CMV: cytomegalovirus; EBV: Epstein-Barr virus; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HSCT: hematopoietic stem cell transplantation; HZ: herpes zoster; IFI: invasive fungal infection; LTBI: latent tuberculous infection; OI: opportunistic infection; PCP: *Pneumocystis jirovecii* pneumonia; RCC: renal cell carcinoma.

**Transparency declaration**

- **Conflict of interest disclosure:** M.R. received personal fees from Roche and Pfizer, and grants and personal fees from Gilead. N.M. received research grants from Swiss National Science Foundation. J.M.A. received personal fees from Pfizer, Astellas and Merck. The remaining authors declare no conflicts of interest (i.e., payment or services from a third party; relevant financial activities outside the submitted work; or patents planned, pending or issued broadly relevant to the submitted work).
- **Funding sources:** This research was partially supported by Plan Nacional de I+D+I 2013-2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Spanish Ministry of Economy and Competitiveness, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0002 and 0008) - co-financed by the European Development Regional Fund (EDRF) "A way to achieve Europe". M.F.R. holds a clinical research contract "Juan Rodés" (JR14/00036) from the Instituto de Salud Carlos III, Spanish Ministry of Economy and Competitiveness.

## References

1. Fernández-Ruiz M, Meije Y, Manuel O, Akan H, Carratalà J, Aguado JM, Delaloye J. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the Safety of targeted and biological therapies: an Infectious Diseases perspective: Introduction. *Clin Microbiol Infect* 2018 (in press).
2. Jain P, Kantarjian H, Cortes J. Chronic myeloid leukemia: overview of new agents and comparative analysis. *Curr Treat Options Oncol* 2013;14:127-43.
3. Hantschel O, Rix U, Superti-Furga G. Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. *Leuk Lymphoma* 2008;49:615-9.
4. Deininger MW, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with imatinib. *Pharmacol Rev* 2003;55:401-23.
5. Seggewiss R, Lore K, Greiner E, Magnusson MK, Price DA, Douek DC, et al. Imatinib inhibits T-cell receptor-mediated T-cell proliferation and activation in a dose-dependent manner. *Blood* 2005;105:2473-9.
6. Appel S, Boehmler AM, Grunebach F, Muller MR, Rupf A, Weck MM, et al. Imatinib mesylate affects the development and function of dendritic cells generated from CD34+ peripheral blood progenitor cells. *Blood* 2004;103:538-44.
7. Chen J, Schmitt A, Giannopoulos K, Chen B, Rojewski M, Dohner H, et al. Imatinib impairs the proliferation and function of CD4+CD25+ regulatory T cells in a dose-dependent manner. *Int J Oncol* 2007;31:1133-9.
8. Chen J, Schmitt A, Chen B, Rojewski M, Rubeler V, Fei F, et al. Nilotinib hampers the proliferation and function of CD8+ T lymphocytes through inhibition of T cell receptor signalling. *J Cell Mol Med* 2008;12:2107-18.
9. Fei F, Yu Y, Schmitt A, Rojewski MT, Chen B, Greiner J, et al. Dasatinib exerts an immunosuppressive effect on CD8+ T cells specific for viral and leukemia antigens. *Exp Hematol* 2008;36:1297-308.

10. de Lavallade H, Khoder A, Hart M, Sarvaria A, Sekine T, Alsuliman A, et al. Tyrosine kinase inhibitors impair B-cell immune responses in CML through off-target inhibition of kinases important for cell signaling. *Blood* 2013;122:227-38.
11. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003;348:994-1004.
12. Breccia M, Girmenia C, Latagliata R, Loglisci G, Santopietro M, Federico V, et al. Low incidence rate of opportunistic and viral infections during imatinib treatment in chronic myeloid leukemia patients in early and late chronic phase. *Mediterr J Hematol Infect Dis* 2011;3:e2011021.
13. Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol* 2006;24:460-6.
14. Daver N, Thomas D, Ravandi F, Cortes J, Garris R, Jabbour E, et al. Final report of a phase II study of imatinib mesylate with hyper-CVAD for the front-line treatment of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Haematologica* 2015;100:653-61.
15. Ikeda K, Shiga Y, Takahashi A, Kai T, Kimura H, Takeyama K, et al. Fatal hepatitis B virus reactivation in a chronic myeloid leukemia patient during imatinib mesylate treatment. *Leuk Lymphoma* 2006;47:155-7.
16. Thia TJ, Tan HH, Chuah TH, Chow WC, Lui HF. Imatinib mesylate-related fatal acute hepatic failure in a patient with chronic myeloid leukaemia and chronic hepatitis B infection. *Singapore Med J* 2008;49:e86-9.
17. Kang BW, Lee SJ, Moon JH, Kim SN, Chae YS, Kim JG, et al. Chronic myeloid leukemia patient manifesting fatal hepatitis B virus reactivation during treatment with imatinib rescued by liver transplantation: case report and literature review. *Int J Hematol* 2009;90:383-7.
18. Lai GM, Yan SL, Chang CS, Tsai CY. Hepatitis B reactivation in chronic myeloid leukemia patients receiving tyrosine kinase inhibitor. *World J Gastroenterol* 2013;19:1318-21.

19. Walker EJ, Simko JP, Ko AH. Hepatitis B viral reactivation secondary to imatinib treatment in a patient with gastrointestinal stromal tumor. *Anticancer Res* 2014;34:3629-34.
20. Lakhani S, Davidson L, Priebat DA, Sherker AH. Reactivation of chronic hepatitis B infection related to imatinib mesylate therapy. *Hepatol Int* 2008;2:498-9.
21. Mattiuzzi GN, Cortes JE, Talpaz M, Reuben J, Rios MB, Shan J, et al. Development of Varicella-Zoster virus infection in patients with chronic myelogenous leukemia treated with imatinib mesylate. *Clin Cancer Res* 2003;9:976-80.
22. Durosini MA, Ogbe PO, Salawu L, Oyekunle AA. Herpes zoster complicating imatinib mesylate for gastrointestinal stromal tumour. *Singapore Med J* 2007;48:e16-8.
23. Leguay T, Foucaud C, Parrens M, Fitoussi O, Bouabdallah K, Belaud-Rotureau MA, et al. EBV-positive lymphoproliferative disease with medullary, splenic and hepatic infiltration after imatinib mesylate therapy for chronic myeloid leukemia. *Leukemia* 2007;21:2208-10.
24. Takashima M, Igaki N, Matsuda T, Ohyama M, Kanda S, Tamada F, et al. Malignant gastrointestinal stromal tumor of the small intestine complicated with pulmonary tuberculosis during treatment with imatinib mesylate. *Intern Med* 2005;44:114-9.
25. Daniels JM, Vonk-Noordegraaf A, Janssen JJ, Postmus PE, van Altena R. Tuberculosis complicating imatinib treatment for chronic myeloid leukaemia. *Eur Respir J* 2009;33:670-2.
26. Senn L, Kovacovics T, Tarr PE, Meylan P. Peritoneal tuberculosis after imatinib therapy. *Arch Intern Med* 2009;169:312-3.
27. Lin JT, Lee MY, Hsiao LT, Yang MH, Chao TC, Chen PM, et al. Pulmonary nocardiosis in a patient with CML relapse undergoing imatinib therapy after bone marrow transplantation. *Ann Hematol* 2004;83:444-6.
28. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2010;362:2251-9.
29. Larson RA, Hochhaus A, Hughes TP, Clark RE, Etienne G, Kim DW, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. *Leukemia* 2012;26:2197-203.



30. Koren-Michowitz M, le Coutre P, Duyster J, Scheid C, Panayiotidis P, Prejzner W, et al. Activity and tolerability of nilotinib: a retrospective multicenter analysis of chronic myeloid leukemia patients who are imatinib resistant or intolerant. *Cancer* 2010;116:4564-72.
31. Reinwald M, Boch T, Hofmann WK, Buchheidt D. Risk of Infectious Complications in Hemato-Oncological Patients Treated with Kinase Inhibitors. *Biomark Insights* 2015;10:55-68.
32. Rodriguez GH, Ahmed SI, Al-akhrass F, Rallapalli V, Safdar A. Characteristics of, and risk factors for, infections in patients with cancer treated with dasatinib and a brief review of other complications. *Leuk Lymphoma* 2012;53:1530-5.
33. Lilly MB, Ottmann OG, Shah NP, Larson RA, Reiffers JJ, Ehninger G, et al. Dasatinib 140 mg once daily versus 70 mg twice daily in patients with Ph-positive acute lymphoblastic leukemia who failed imatinib: Results from a phase 3 study. *Am J Hematol* 2010;85:164-70.
34. Prestes DP, Arbona E, Nevett-Fernandez A, Woolley AE, Ho VT, Koo S, et al. Dasatinib Use and Risk of Cytomegalovirus Reactivation After Allogeneic Hematopoietic-Cell Transplantation. *Clin Infect Dis* 2017;65:510-3.
35. Davalos F, Chaucer B, Zafar W, Salman S, Nfonoyim J. Dasatinib-Induced CMV Hepatitis in an Immunocompetent Patient: A Rare Complication of a Common Drug. *Transl Oncol* 2016;9:248-50.
36. Aldoss I, Gaal K, Al Malki MM, Ali H, Nakamura R, Forman SJ, et al. Dasatinib-Induced Colitis after Allogeneic Stem Cell Transplantation for Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia. *Biol Blood Marrow Transplant* 2016;22:1900-3.
37. Yassin MA, Nashwan AJ, Soliman AT, Yousif A, Moustafa A, AlBattah A, et al. Cytomegalovirus-induced Hemorrhagic Colitis in a Patient with Chronic Myeloid Leukemia (Chronic Phase) on Dasatinib as an Upfront Therapy. *Clin Med Insights Case Rep* 2015;8:77-81.
38. Knoll BM, Seiter K. Infections in patients on BCR-ABL tyrosine kinase inhibitor therapy: cases and review of the literature. *Infection* 2018.
39. Sillaber C, Herrmann H, Bennett K, Rix U, Baumgartner C, Bohm A, et al. Immunosuppression and atypical infections in CML patients treated with dasatinib at 140 mg daily. *Eur J Clin Invest* 2009;39:1098-109.

40. Chang H, Hung YS, Chou WC. *Pneumocystis jiroveci* pneumonia in patients receiving dasatinib treatment. *Int J Infect Dis* 2014;25:165-7.
41. Ando T, Kojima K, Isoda H, Eguchi Y, Honda T, Ishigami M, et al. Reactivation of resolved infection with the hepatitis B virus immune escape mutant G145R during dasatinib treatment for chronic myeloid leukemia. *Int J Hematol* 2015;102:379-82.
42. Garcia-Munoz R, Galar A, Moreno C, Rodriguez-Otero P, Panizo-Morgado E, Ponz-Sarvise M, et al. Parvovirus B19 acute infection and a reactivation of cytomegalovirus and herpesvirus 6 in a chronic myeloid leukemia patient during treatment with dasatinib (BMS-354825). *Leuk Lymphoma* 2007;48:2461-4.
43. Gambacorti-Passerini C, Cortes JE, Lipton JH, Dmoszynska A, Wong RS, Rossiev V, et al. Safety of bosutinib versus imatinib in the phase 3 BELA trial in newly diagnosed chronic phase chronic myeloid leukemia. *Am J Hematol* 2014;89:947-53.
44. Brummendorf TH, Cortes JE, de Souza CA, Guilhot F, Duvillie L, Pavlov D, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukaemia: results from the 24-month follow-up of the BELA trial. *Br J Haematol* 2015;168:69-81.
45. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med* 2013;369:1783-96.
46. Jabbour E, Kantarjian H, Cortes J. Use of second- and third-generation tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia: an evolving treatment paradigm. *Clin Lymphoma Myeloma Leuk* 2015;15:323-34.
47. Garg RJ, Kantarjian H, O'Brien S, Quintas-Cardama A, Faderl S, Estrov Z, et al. The use of nilotinib or dasatinib after failure to 2 prior tyrosine kinase inhibitors: long-term follow-up. *Blood* 2009;114:4361-8.
48. Giles FJ, Abruzzese E, Rosti G, Kim DW, Bhatia R, Bosly A, et al. Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. *Leukemia* 2010;24:1299-301.

49. Ibrahim AR, Paliompeis C, Bua M, Milojkovic D, Szydlo R, Khorashad JS, et al. Efficacy of tyrosine kinase inhibitors (TKIs) as third-line therapy in patients with chronic myeloid leukemia in chronic phase who have failed 2 prior lines of TKI therapy. *Blood* 2010;116:5497-500.
50. Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. *Blood* 2012;119:3403-12.
51. Maertens J, Cesaro S, Maschmeyer G, Einsele H, Donnelly JP, Alanio A, et al. ECIL guidelines for preventing *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. *J Antimicrob Chemother* 2016;71:2397-404.
52. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol* 2017.
53. Haasbach E, Hartmayer C, Planz O. Combination of MEK inhibitors and oseltamivir leads to synergistic antiviral effects after influenza A virus infection in vitro. *Antiviral Res* 2013;98:319-24.
54. Lieske NV, Tonby K, Kvale D, Dyrhol-Riise AM, Tasken K. Targeting Tuberculosis and HIV Infection-Specific Regulatory T Cells with MEK/ERK Signaling Pathway Inhibitors. *PLoS One* 2015;10:e0141903.
55. Du SL, Yuan X, Zhan S, Tang LJ, Tong CY. Trametinib, a novel MEK kinase inhibitor, suppresses lipopolysaccharide-induced tumor necrosis factor (TNF)-alpha production and endotoxin shock. *Biochem Biophys Res Commun* 2015;458:667-73.
56. Liu W, Yang R, Payne AS, Schowalter RM, Spurgeon ME, Lambert PF, et al. Identifying the Target Cells and Mechanisms of Merkel Cell Polyomavirus Infection. *Cell Host Microbe* 2016;19:775-87.
57. Cowan N, Keiser J. Repurposing of anticancer drugs: in vitro and in vivo activities against *Schistosoma mansoni*. *Parasit Vectors* 2015;8:417.
58. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;364:2507-16.
59. Cohen DN, Lawson SK, Shaver AC, Du L, Nguyen HP, He Q, et al. Contribution of Beta-HPV Infection and UV Damage to Rapid-Onset Cutaneous Squamous Cell Carcinoma during BRAF-Inhibition Therapy. *Clin Cancer Res* 2015;21:2624-34.

60. Frouin E, Guillot B, Larrieux M, Tempier A, Boule N, Foulongne V, et al. Cutaneous epithelial tumors induced by vemurafenib involve the MAPK and Pi3KCA pathways but not HPV nor HPyV viral infection. *PLoS One* 2014;9:e110478.
61. McArthur GA, Chapman PB, Robert C, Larkin J, Haanen JB, Dummer R, et al. Safety and efficacy of vemurafenib in BRAF(V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. *Lancet Oncol* 2014;15:323-32.
62. Ascierto PA, McArthur GA, Dreno B, Atkinson V, Liskay G, Di Giacomo AM, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2016;17:1248-60.
63. Schilling B, Sonderrmann W, Zhao F, Griewank KG, Livingstone E, Sucker A, et al. Differential influence of vemurafenib and dabrafenib on patients' lymphocytes despite similar clinical efficacy in melanoma. *Ann Oncol* 2014;25:747-53.
64. Sonderrmann W, Griewank KG, Schilling B, Livingstone E, Leyh JC, Rompoti N, et al. Corticosteroids augment BRAF inhibitor vemurafenib induced lymphopenia and risk of infection. *PLoS One* 2015;10:e0124590.
65. Marsico JG, Rodriguez R, Muller J, Jorger M. Vemurafenib-related sterile scrotal abscess in a patient with BRAFV600K-mutant advanced melanoma mimicking distant metastasis. *J Cancer Res Ther* 2015;11:647.
66. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012;380:358-65.
67. Anforth RM, Blumetti TC, Kefford RF, Sharma R, Scolyer RA, Kossard S, et al. Cutaneous manifestations of dabrafenib (GSK2118436): a selective inhibitor of mutant BRAF in patients with metastatic melanoma. *Br J Dermatol* 2012;167:1153-60.
68. Vergote V, Dierickx D, Janssens A, Verhoef G, Tousseyn T, Vandenberghe P, et al. Rapid and complete hematological response of refractory hairy cell leukemia to the BRAF inhibitor dabrafenib. *Ann Hematol* 2014;93:2087-9.

69. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012;367:1694-703.
70. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 2014;371:1877-88.
71. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med* 2015;372:30-9.
72. Coleman RL, Sill MW, Thaker PH, Bender DP, Street D, McGuire WP, et al. A phase II evaluation of selumetinib (AZD6244, ARRY-142886), a selective MEK-1/2 inhibitor in the treatment of recurrent or persistent endometrial cancer: an NRG Oncology/Gynecologic Oncology Group study. *Gynecol Oncol* 2015;138:30-5.
73. Liu M, Yang X, Liu J, Zhao B, Cai W, Li Y, et al. Efficacy and safety of BRAF inhibition alone versus combined BRAF and MEK inhibition in melanoma: a meta-analysis of randomized controlled trials. *Oncotarget* 2017.
74. Cebollero A, Puertolas T, Pajares I, Calera L, Anton A. Comparative safety of BRAF and MEK inhibitors (vemurafenib, dabrafenib and trametinib) in first-line therapy for BRAF-mutated metastatic melanoma. *Mol Clin Oncol* 2016;5:458-62.
75. Welsh SJ, Corrie PG. Management of BRAF and MEK inhibitor toxicities in patients with metastatic melanoma. *Ther Adv Med Oncol* 2015;7:122-36.
76. Herman SE, Gordon AL, Hertlein E, Ramanunni A, Zhang X, Jaglowski S, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* 2011;117:6287-96.
77. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993;72:279-90.
78. Sun C, Tian X, Lee YS, Gunti S, Lipsky A, Herman SE, et al. Partial reconstitution of humoral immunity and fewer infections in patients with chronic lymphocytic leukemia treated with ibrutinib. *Blood* 2015;126:2213-9.

79. Byrd JC, Brown JR, O'Brien S, Barrientos JC, Kay NE, Reddy NM, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med* 2014;371:213-23.
80. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2013;369:32-42.
81. Chanan-Khan A, Cramer P, Demirkan F, Fraser G, Silva RS, Grosicki S, et al. Ibrutinib combined with bendamustine and rituximab compared with placebo, bendamustine, and rituximab for previously treated chronic lymphocytic leukaemia or small lymphocytic lymphoma (HELIOS): a randomised, double-blind, phase 3 study. *Lancet Oncol* 2016;17:200-11.
82. Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Ghia P, et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. *N Engl J Med* 2015;373:2425-37.
83. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med* 2013;369:507-16.
84. Dreyling M, Jurczak W, Jerkeman M, Silva RS, Rusconi C, Trneny M, et al. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: an international, randomised, open-label, phase 3 study. *Lancet* 2016;387:770-8.
85. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med* 2015;372:1430-40.
86. Okamoto K, Proia LA, Demarais PL. Disseminated Cryptococcal Disease in a Patient with Chronic Lymphocytic Leukemia on Ibrutinib. *Case Rep Infect Dis* 2016;2016:4642831.
87. Stankowicz M, Banaszynski M, Crawford R. Cryptococcal infections in two patients receiving ibrutinib therapy for chronic lymphocytic leukemia. *J Oncol Pharm Pract* 2018;1078155217752078.
88. Messina JA, Maziarz EK, Spec A, Kontoyiannis DP, Perfect JR. Disseminated Cryptococcosis With Brain Involvement in Patients With Chronic Lymphoid Malignancies on Ibrutinib. *Open Forum Infect Dis* 2017;4:ofw261.
89. Ahn IE, Jerussi T, Farooqui M, Tian X, Wiestner A, Gea-Banacloche J. Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood* 2016;128:1940-3.

90. Lee R, Nayernama A, Jones SC, Wroblewski T, Waldron PE. Ibrutinib-associated *Pneumocystis jirovecii* pneumonia. *Am J Hematol* 2017.
91. JM P. Integrated infectious toxicity analysis of the BTK inhibitor ibrutinib. *Journal of Clinical Oncology*, s 2016 2016;supl. 15 34 (May 2016).
92. Arthurs B, Wunderle K, Hsu M, Kim S. Invasive aspergillosis related to ibrutinib therapy for chronic lymphocytic leukemia. *Respir Med Case Rep* 2017;21:27-9.
93. Gaye E, Le Bot A, Talarmin JP, Le Calloch R, Belaz S, Dupont M, et al. Cerebral aspergillosis: An emerging opportunistic infection in patients receiving ibrutinib for chronic lymphocytic leukemia? *Med Mal Infect* 2018.
94. Chan TS, Au-Yeung R, Chim CS, Wong SC, Kwong YL. Disseminated fusarium infection after ibrutinib therapy in chronic lymphocytic leukaemia. *Ann Hematol* 2017;96:871-2.
95. Lutz M, Schulze AB, Rebber E, Wiebe S, Zoubi T, Grauer OM, et al. Progressive multifocal leukoencephalopathy after ibrutinib therapy for chronic lymphocytic leukemia. *Cancer Res Treat* 2017;49:548-52.
96. Raisch DW, Rafi JA, Chen C, Bennett CL. Detection of cases of progressive multifocal leukoencephalopathy associated with new biologicals and targeted cancer therapies from the FDA's adverse event reporting system. *Expert Opin Drug Saf* 2016;15:1003-11.
97. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with ibrutinib and other small Molecule kinase inhibitors targeting immune signaling pathways. *Clin Infect Dis* 2018;66:140-8.
98. Brown JR. How I treat CLL patients with ibrutinib. *Blood* 2018;131:379-86.
99. Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, et al. Acalabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med* 2016;374:323-32.
100. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* 2003;3:317-30.
101. Samuels Y, Ericson K. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* 2006;18:77-82.

102. Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, Johnson AJ, et al. CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. *Blood* 2011;117:591-4.
103. Zydelig Summary of Product Characteristics N.
104. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood* 2011;118:3603-12.
105. Xie S, Chen M, Yan B, He X, Chen X, Li D. Identification of a role for the PI3K/AKT/mTOR signaling pathway in innate immune cells. *PLoS One* 2014;9:e94496.
106. Rafii S, Roda D, Geuna E, Jimenez B, Rihawi K, Capelan M, et al. Higher Risk of Infections with PI3K-AKT-mTOR Pathway Inhibitors in Patients with Advanced Solid Tumors on Phase I Clinical Trials. *Clin Cancer Res* 2015;21:1869-76.
107. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2014;370:997-1007.
108. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* 2014;370:1008-18.
109. Sehn LH HM, Jurczak W, Brown JR, Barr PM, Catalano J, et al. A retrospective analysis of *Pneumocystis jirovecii* pneumonia infection in patients receiving idelalisib in Clinical Trials. Paper presented at: American Society of Hematology (ASH) 2016;03-06 December, 2016; San Diego, CA.
110. Sharman JP SG, Jurczak W, et al. Temporal Profiles of Lymphocyte Subsets and the Correlation with Infectious Events in Idelalisib-Treated Patients. *Blood* 2016;128::5583.
111. Jones JA, Robak T, Brown JR, Awan FT, Badoux X, Coutre S, et al. Efficacy and safety of idelalisib in combination with ofatumumab for previously treated chronic lymphocytic leukaemia: an open-label, randomised phase 3 trial. *Lancet Haematol* 2017;4:e114-e26.
112. Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013;19:202-8.
113. Fischer K, Cramer P, Busch R, Stilgenbauer S, Bahlo J, Schweighofer CD, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a



- multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol* 2011;29:3559-66.
114. Hardwick JM, Soane L. Multiple functions of BCL-2 family proteins. *Cold Spring Harb Perspect Biol* 2013;5.
115. Zhan Y, Carrington EM, Ko HJ, Vikstrom IB, Oon S, Zhang JG, et al. Bcl-2 antagonists kill plasmacytoid dendritic cells from lupus-prone mice and dampen interferon-alpha production. *Arthritis Rheumatol* 2015;67:797-808.
116. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med* 2016;374:311-22.
117. Stilgenbauer S, Eichhorst B, Schetelig J, Coutre S, Seymour JF, Munir T, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol* 2016;17:768-78.
118. Clinical Practice Guideline Forefoot Disorders P, Thomas JL, Blitch ELt, Chaney DM, Dinucci KA, Eickmeier K, et al. Diagnosis and treatment of forefoot disorders. Section 4. Tailor's bunion. *J Foot Ankle Surg* 2009;48:257-63.
119. Agarwal SK, Salem AH, Danilov AV, Hu B, Puvvada S, Gutierrez M, et al. Effect of ketoconazole, a strong CYP3A inhibitor, on the pharmacokinetics of venetoclax, a BCL-2 inhibitor, in patients with non-Hodgkin lymphoma. *Br J Clin Pharmacol* 2017;83:846-54.
120. Khwaja A. The role of Janus kinases in haemopoiesis and haematological malignancy. *Br J Haematol* 2006;134:366-84.
121. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, et al. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. *Science* 1995;270:797-800.
122. Macchi P, Villa A, Gilliani S, Sacco MG, Frattini A, Porta F, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 1995;377:65-8.
123. O'Shea JJ, Kontzias A, Yamaoka K, Tanaka Y, Laurence A. Janus kinase inhibitors in autoimmune diseases. *Ann Rheum Dis* 2013;72 Suppl 2:ii111-ii5.
124. Casanova JL, Holland SM, Notarangelo LD. Inborn errors of human JAKs and STATs. *Immunity* 2012;36:515-28.

125. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood* 2005;106:2162-8.
126. Staerk J, Constantinescu SN. The JAK-STAT pathway and hematopoietic stem cells from the JAK2 V617F perspective. *JAKSTAT* 2012;1:184-90.
127. Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E, et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood* 2005;106:3377-9.
128. Harrison C, Kiladjian JJ, Al Ali HK, Gisslinger H, Waltzman R, Stalbovska V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* 2012;366:787-98.
129. Vannucchi AM, Kiladjian JJ, Grieshammer M, Masszi T, Durrant S, Passamonti F, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Engl J Med* 2015;372:426-35.
130. Burmester GR, Blanco R, Charles-Schoeman C, Wollenhaupt J, Zerbini C, Benda B, et al. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 2013;381:451-60.
131. Taylor PC, Keystone EC, van der HD, Weinblatt ME, Del Carmen ML, Reyes GJ, et al. Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis. *N Engl J Med* 2017;376:652-62.
132. Vincenti F, Tedesco SH, Busque S, O'Connell P, Friedewald J, Cibrik D, et al. Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. *Am J Transplant* 2012;12:2446-56.
133. Vincenti F, Silva HT, Busque S, O'Connell PJ, Russ G, Budde K, et al. Evaluation of the effect of tofacitinib exposure on outcomes in kidney transplant patients. *Am J Transplant* 2015;15:1644-53.
134. Bachelez H, van de Kerkhof PC, Strohal R, Kubanov A, Valenzuela F, Lee JH, et al. Tofacitinib versus etanercept or placebo in moderate-to-severe chronic plaque psoriasis: a phase 3 randomised non-inferiority trial. *Lancet* 2015;386:552-61.

135. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia* 2015;29:2062-8.
136. Hurwitz HI, Uppal N, Wagner SA, Bendell JC, Beck JT, Wade SM, 3rd, et al. Randomized, Double-Blind, Phase II Study of Ruxolitinib or Placebo in Combination With Capecitabine in Patients With Metastatic Pancreatic Cancer for Whom Therapy With Gemcitabine Has Failed. *J Clin Oncol* 2015;33:4039-47.
137. Parampalli YS, Stubig T, Cornez I, Alchalby H, Schonberg K, Rudolph J, et al. JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. *Br J Haematol* 2015;169:824-33.
138. Keohane C, Kordasti S, Seidl T, Perez AP, Thomas NS, Harrison CN, et al. JAK inhibition induces silencing of T Helper cytokine secretion and a profound reduction in T regulatory cells. *Br J Haematol* 2015;171:60-73.
139. Heine A, Held SA, Daecke SN, Wallner S, Yajnanarayana SP, Kurts C, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood* 2013;122:1192-202.
140. Schonberg K, Rudolph J, Vonnahme M, Parampalli YS, Cornez I, Hejazi M, et al. JAK Inhibition Impairs NK Cell Function in Myeloproliferative Neoplasms. *Cancer Res* 2015;75:2187-99.
141. Maeshima K, Yamaoka K, Kubo S, Nakano K, Iwata S, Saito K, et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-gamma and interleukin-17 production by human CD4+ T cells. *Arthritis Rheum* 2012;64:1790-8.
142. Ghoreschi K, Jesson MI, Li X, Lee JL, Ghosh S, Alsup JW, et al. Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). *J Immunol* 2011;186:4234-43.
143. Cervantes F, Vannucchi AM, Kiladjan JJ, Al Ali HK, Sirulnik A, Stalbovska V, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood* 2013;122:4047-53.
144. Harrison CN, Vannucchi AM, Kiladjan JJ, Al Ali HK, Gisslinger H, Knoops L, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. *Leukemia* 2016;30:1701-7.

145. Jung CW, Shih LY, Xiao Z, Jie J, Hou HA, Du X, et al. Efficacy and safety of ruxolitinib in Asian patients with myelofibrosis. *Leuk Lymphoma* 2015;56:2067-74.
146. Al Ali HK, Griesshammer M, le Coutre P, Waller CF, Liberati AM, Schafhausen P, et al. 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* 2016;101:1065-73.
147. Pemmaraju N, Kantarjian H, Kadia T, Cortes J, Borthakur G, Newberry K, et al. A phase I/II study of the Janus kinase (JAK)1 and 2 inhibitor ruxolitinib in patients with relapsed or refractory acute myeloid leukemia. *Clin Lymphoma Myeloma Leuk* 2015;15:171-6.
148. Shanavas M, Popat U, Michaelis LC, Fauble V, McLornan D, Klisovic R, et al. Outcomes of Allogeneic Hematopoietic Cell Transplantation in Patients with Myelofibrosis with Prior Exposure to Janus Kinase 1/2 Inhibitors. *Biol Blood Marrow Transplant* 2016;22:432-40.
149. Caocci G, Murgia F, Podda L, Solinas A, Atzeni S, La Nasa G. Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. *Leukemia* 2014;28:225-7.
150. Perricone G, Vinci M, Pungolino E. Occult hepatitis B infection reactivation after ruxolitinib therapy. *Dig Liver Dis* 2017;49:719.
151. Wysham NG, Sullivan DR, Allada G. An opportunistic infection associated with ruxolitinib, a novel janus kinase 1,2 inhibitor. *Chest* 2013;143:1478-9.
152. Lee SC, Feenstra J, Georghiou PR. *Pneumocystis jiroveci* pneumonitis complicating ruxolitinib therapy. *BMJ Case Rep* 2014;2014.
153. Goldberg RA, Reichel E, Oshry LJ. Bilateral toxoplasmosis retinitis associated with ruxolitinib. *N Engl J Med* 2013;369:681-3.
154. Colomba C, Rubino R, Siracusa L, Lalicata F, Trizzino M, Titone L, et al. Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. *BMC Res Notes* 2012;5:552.
155. Wathes R, Moule S, Milojkovic D. Progressive multifocal leukoencephalopathy associated with ruxolitinib. *N Engl J Med* 2013;369:197-8.

156. Fleischmann R, Kremer J, Cush J, Schulze-Koops H, Connell CA, Bradley JD, et al. Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis. *N Engl J Med* 2012;367:495-507.
157. Cohen S, Radominski SC, Gomez-Reino JJ, Wang L, Krishnaswami S, Wood SP, et al. Analysis of infections and all-cause mortality in phase II, phase III, and long-term extension studies of tofacitinib in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:2924-37.
158. Strand V, Ahadieh S, French J, Geier J, Krishnaswami S, Menon S, et al. Systematic review and meta-analysis of serious infections with tofacitinib and biologic disease-modifying antirheumatic drug treatment in rheumatoid arthritis clinical trials. *Arthritis Res Ther* 2015;17:362.
159. Charles-Schoeman C, Burmester G, Nash P, Zerbini CA, Soma K, Kwok K, et al. Efficacy and safety of tofacitinib following inadequate response to conventional synthetic or biological disease-modifying antirheumatic drugs. *Ann Rheum Dis* 2016;75:1293-301.
160. Gladman D, Rigby W, Azevedo VF, Behrens F, Blanco R, Kaszuba A, et al. Tofacitinib for Psoriatic Arthritis in Patients with an Inadequate Response to TNF Inhibitors. *N Engl J Med* 2017;377:1525-36.
161. Mease P, Hall S, FitzGerald O, van der Heijde D, Merola JF, Avila-Zapata F, et al. Tofacitinib or Adalimumab versus Placebo for Psoriatic Arthritis. *N Engl J Med* 2017;377:1537-50.
162. Winthrop KL, Park SH, Gul A, Cardiel MH, Gomez-Reino JJ, Tanaka Y, et al. Tuberculosis and other opportunistic infections in tofacitinib-treated patients with rheumatoid arthritis. *Ann Rheum Dis* 2016;75:1133-8.
163. Singh JA, Saag KG, Bridges SL, Jr., Akl EA, Bannuru RR, Sullivan MC, et al. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol* 2016;68:1-26.
164. Moore CA, Iasella CJ, Venkataramanan R, Lakkis FG, Smith RB, McDyer JF, et al. Janus kinase inhibition for immunosuppression in solid organ transplantation: Is there a role in complex immunologic challenges? *Hum Immunol* 2017;78:64-71.
165. Genovese MC, Kremer J, Zamani O, Ludivico C, Krogulec M, Xie L, et al. Baricitinib in Patients with Refractory Rheumatoid Arthritis. *N Engl J Med* 2016;374:1243-52.

166. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009;9:550-62.
167. Jacinto E, Hall MN. Tor signalling in bugs, brain and brawn. *Nat Rev Mol Cell Biol* 2003;4:117-26.
168. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002;110:163-75.
169. Perl A. mTOR activation is a biomarker and a central pathway to autoimmune disorders, cancer, obesity, and aging. *Ann N Y Acad Sci* 2015;1346:33-44.
170. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 2004;4:335-48.
171. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002;10:151-62.
172. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;18:1926-45.
173. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol* 2003;13:1259-68.
174. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. *Trends Mol Med* 2007;13:433-42.
175. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* 2008;412:179-90.
176. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science* 1996;273:239-42.
177. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002;8:128-35.
178. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev* 2008;22:239-51.

179. Meric-Bernstam F, Gonzalez-Angulo AM. Targeting the mTOR signaling network for cancer therapy. *J Clin Oncol* 2009;27:2278-87.
180. Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, et al. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol* 2010;28:245-55.
181. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangiomyomatosis. *N Engl J Med* 2008;358:140-51.
182. Herry I, Neukirch C, Debray MP, Mignon F, Crestani B. Dramatic effect of sirolimus on renal angiomyolipomas in a patient with tuberous sclerosis complex. *Eur J Intern Med* 2007;18:76-7.
183. Stallone G, Schena A, Infante B, Di Paolo S, Loverre A, Maggio G, et al. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N Engl J Med* 2005;352:1317-23.
184. Campistol JM, Gutierrez-Dalmau A, Torregrosa JV. Conversion to sirolimus: a successful treatment for posttransplantation Kaposi's sarcoma. *Transplantation* 2004;77:760-2.
185. Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol* 2011;29:4452-61.
186. Kim WY, Kaelin WG. Role of VHL gene mutation in human cancer. *J Clin Oncol* 2004;22:4991-5004.
187. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007;356:2271-81.
188. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 2008;372:449-56.
189. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. *Cancer* 2010;116:4256-65.
190. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, et al. Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* 2011;364:514-23.

191. Baselga J, Campone M, Piccart M, Burris HA, 3rd, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2012;366:520-9.
192. Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2013;381:125-32.
193. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol* 2009;27:3822-9.
194. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, et al. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 2005;23:5294-304.
195. Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, et al. CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. *Cancer* 2005;104:1045-8.
196. Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Dittrich C, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol* 2005;23:5314-22.
197. An P, Mukherjee O, Chanda P, Yao L, Engelman CD, Huang CH, et al. The challenge of detecting epistasis (G x G interactions): Genetic Analysis Workshop 16. *Genet Epidemiol* 2009;33 Suppl 1:S58-67.
198. Fantus D, Thomson AW. Evolving perspectives of mTOR complexes in immunity and transplantation. *Am J Transplant* 2015;15:891-902.
199. Havenith SH, Yong SL, van Donselaar-van der Pant KA, van Lier RA, ten Berge IJ, Bemelman FJ. Everolimus-treated renal transplant recipients have a more robust CMV-specific CD8+ T-cell response compared with cyclosporine- or mycophenolate-treated patients. *Transplantation* 2013;95:184-91.
200. Pascual J, Royuela A, Fernandez AM, Herrero I, Delgado JF, Sole A, et al. Role of mTOR inhibitors for the control of viral infection in solid organ transplant recipients. *Transpl Infect Dis* 2016;18:819-31.



201. Mallat SG, Tanios BY, Itani HS, Lotfi T, McMullan C, Gabardi S, et al. CMV and BKPyV Infections in Renal Transplant Recipients Receiving an mTOR Inhibitor-Based Regimen Versus a CNI-Based Regimen: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *Clin J Am Soc Nephrol* 2017;12:1321-36.
202. Pollizzi KN, Powell JD. Regulation of T cells by mTOR: the known knowns and the known unknowns. *Trends Immunol* 2015;36:13-20.
203. Turner AP, Shaffer VO, Araki K, Martens C, Turner PL, Gangappa S, et al. Sirolimus enhances the magnitude and quality of viral-specific CD8+ T-cell responses to vaccinia virus vaccination in rhesus macaques. *Am J Transplant* 2011;11:613-8.
204. Limon JJ, Fruman DA. Akt and mTOR in B Cell Activation and Differentiation. *Front Immunol* 2012;3:228.
205. van Gelder T, ter Meulen CG, Hene R, Weimar W, Hoitsma A. Oral ulcers in kidney transplant recipients treated with sirolimus and mycophenolate mofetil. *Transplantation* 2003;75:788-91.
206. Yang H, Wang X, Zhang Y, Liu H, Liao J, Shao K, et al. Modulation of TSC-mTOR signaling on immune cells in immunity and autoimmunity. *J Cell Physiol* 2014;229:17-26.
207. Gomez-Cambronero J. Rapamycin inhibits GM-CSF-induced neutrophil migration. *FEBS Lett* 2003;550:94-100.
208. Gomez-Cambronero J, Horn J, Paul CC, Baumann MA. Granulocyte-macrophage colony-stimulating factor is a chemoattractant cytokine for human neutrophils: involvement of the ribosomal p70 S6 kinase signaling pathway. *J Immunol* 2003;171:6846-55.
209. Lorne E, Zhao X, Zmijewski JW, Liu G, Park YJ, Tsuruta Y, et al. Participation of mammalian target of rapamycin complex 1 in Toll-like receptor 2- and 4-induced neutrophil activation and acute lung injury. *Am J Respir Cell Mol Biol* 2009;41:237-45.
210. Liu L, Das S, Losert W, Parent CA. mTORC2 regulates neutrophil chemotaxis in a cAMP- and RhoA-dependent fashion. *Dev Cell* 2010;19:845-57.
211. Squarize CH, Castilho RM, Bugge TH, Gutkind JS. Accelerated wound healing by mTOR activation in genetically defined mouse models. *PLoS One* 2010;5:e10643.

212. Katholnig K, Linke M, Pham H, Hengstschlager M, Weichhart T. Immune responses of macrophages and dendritic cells regulated by mTOR signalling. *Biochem Soc Trans* 2013;41:927-33.
213. Chiarini F, Evangelisti C, McCubrey JA, Martelli AM. Current treatment strategies for inhibiting mTOR in cancer. *Trends Pharmacol Sci* 2015;36:124-35.
214. Kaymakcalan MD, Je Y, Sonpavde G, Galsky M, Nguyen PL, Heng DY, et al. Risk of infections in renal cell carcinoma (RCC) and non-RCC patients treated with mammalian target of rapamycin inhibitors. *Br J Cancer* 2013;108:2478-84.
215. Campbell SB, Walker R, Tai SS, Jiang Q, Russ GR. Randomized controlled trial of sirolimus for renal transplant recipients at high risk for nonmelanoma skin cancer. *Am J Transplant* 2012;12:1146-56.
216. Euvrard S, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, et al. Sirolimus and secondary skin-cancer prevention in kidney transplantation. *N Engl J Med* 2012;367:329-39.
217. Hoogendijk-van den Akker JM, Harden PN, Hoitsma AJ, Proby CM, Wolterbeek R, Bouwes Bavinck JN, et al. Two-year randomized controlled prospective trial converting treatment of stable renal transplant recipients with cutaneous invasive squamous cell carcinomas to sirolimus. *J Clin Oncol* 2013;31:1317-23.
218. Alberu J, Pascoe MD, Campistol JM, Schena FP, Rial Mdel C, Polinsky M, et al. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurin inhibitor-free immunotherapy: 24-month results from the CONVERT trial. *Transplantation* 2011;92:303-10.
219. Campistol JM, Eris J, Oberbauer R, Friend P, Hutchison B, Morales JM, et al. Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J Am Soc Nephrol* 2006;17:581-9.
220. Kaplan B, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. *Transplant Rev (Orlando)* 2014;28:126-33.
221. Budde K, Neumayer HH, Lehne G, Winkler M, Hauser IA, Lison A, et al. Tolerability and steady-state pharmacokinetics of everolimus in maintenance renal transplant patients. *Nephrol Dial Transplant* 2004;19:2606-14.

222. Budde K, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 2011;377:837-47.
223. Bemelman FJ, de Fijter JW, Kers J, Meyer C, Peters-Sengers H, de Maar EF, et al. Early Conversion to Prednisolone/Everolimus as an Alternative Weaning Regimen Associates With Beneficial Renal Transplant Histology and Function: The Randomized-Controlled MECANO Trial. *Am J Transplant* 2017;17:1020-30.
224. de Fijter JW, Holdaas H, Oyen O, Sanders JS, Sundar S, Bemelman FJ, et al. Early Conversion From Calcineurin Inhibitor- to Everolimus-Based Therapy Following Kidney Transplantation: Results of the Randomized ELEVATE Trial. *Am J Transplant* 2016.
225. Pham PT, Pham PC, Danovitch GM, Ross DJ, Gritsch HA, Kendrick EA, et al. Sirolimus-associated pulmonary toxicity. *Transplantation* 2004;77:1215-20.
226. Molas-Ferrer G, Soy-Muner D, Anglada-Martinez H, Riu-Viladoms G, Estefanell-Tejero A, Ribas-Sala J. Interstitial pneumonitis as an adverse reaction to mTOR inhibitors. *Nefrologia* 2013;33:297-300.
227. Qi WX, Huang YJ, Yao Y, Shen Z, Min DL. Incidence and risk of treatment-related mortality with mTOR inhibitors everolimus and temsirolimus in cancer patients: a meta-analysis. *PLoS One* 2013;8:e65166.
228. Knoll GA, Kokolo MB, Mallick R, Beck A, Buenaventura CD, Ducharme R, et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. *BMJ* 2014;349:g6679.
229. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y, Neylan J. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. *Transplantation* 1999;68:1526-32.
230. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet* 2000;356:194-202.

231. Flechner SM, Glyda M, Cockfield S, Grinyo J, Legendre C, Russ G, et al. The ORION study: comparison of two sirolimus-based regimens versus tacrolimus and mycophenolate mofetil in renal allograft recipients. *Am J Transplant* 2011;11:1633-44.
232. Flechner SM, Gurkan A, Hartmann A, Legendre CM, Russ GR, Campistol JM, et al. A randomized, open-label study of sirolimus versus cyclosporine in primary de novo renal allograft recipients. *Transplantation* 2013;95:1233-41.
233. Schena FP, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC, et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation* 2009;87:233-42.
234. Garcia CA, Wu S. Attributable Risk of Infection to mTOR Inhibitors Everolimus and Temsirolimus in the Treatment of Cancer. *Cancer Invest* 2016;34:521-30.
235. Mizuno S, Yamagishi Y, Ebinuma H, Nakamoto N, Katahira M, Sasaki A, et al. Progressive liver failure induced by everolimus for renal cell carcinoma in a 58-year-old male hepatitis B virus carrier. *Clin J Gastroenterol* 2013;6:188-92.
236. Sezgin Goksu S, Bilal S, Coskun HS. Hepatitis B reactivation related to everolimus. *World J Hepatol* 2013;5:43-5.

## Growth factors and cytokines (IGF-1, VEGF...)

