Bcl-2, JAK and mTOR Inhibitors

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mTOR Inhibitors

The mammalian target of rapamycin (mTOR) was discovered through the study of its inhibitor rapamycin, a substance with antitumor and immunosuppressive activity [1] (Fig. 16.1). mTOR associates with a set of proteins to form the mTOR complexes (mTORC) 1 and 2 and acts as the catalytic core. Whereas mTORC1 is efficiently inhibited by rapamycin, mTORC2 is relatively resistant [1]. mTORC1 initiates anabolic processes required for energy storage and cell growth through the promotion of protein, lipid, and nucleotide synthesis. Simultaneously, mTORC1 suppresses catabolism by inhibiting autophagy and degradation of ubiquitinated proteins [2]. mTORC1 activity increases upon nutrient intake and stimulation of growth factor signaling pathways. The latter converge on inhibiting the tuberous sclerosis complex (TSC) 1 and 2, a negative regulator of mTORC1 activity. TSC is exemplary suppressed downstream of insulin growth factor IGF involving PI3K and Akt activation. In addition, signaling pathways such as the Ras/ERK/MAP kinase cascade, involved in cell proliferation, inhibit TSC and thereby stimulate mTORC1 activity. In contrast, DNA damage and the lack of energy and oxygen will prevent mTORC1 activation [2]. Less is known about the role of mTORC2. This complex stimulates proliferation and cell migration and ensures cell survival, most prominently by activating the PI3K/Akt pathway, but also phosphorylates protein kinases involved in cytoskeleton remodeling and ion transport. Just as with mTORC1, mTORC2 activity is stimulated by insulin/PI3K signaling, implying a positive feedback loop. Via Akt signaling, the mTORC1 and 2 pathways are intertwined as mTORC1 inhibits insulin/PI3K mediated mTORC2 activation [2].

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C. Cervera, J. M. Aguado (eds.), *Infectious Complications in Biologic and Targeted Therapies*, https://doi.org/10.1007/978-3-031-11363-5_16

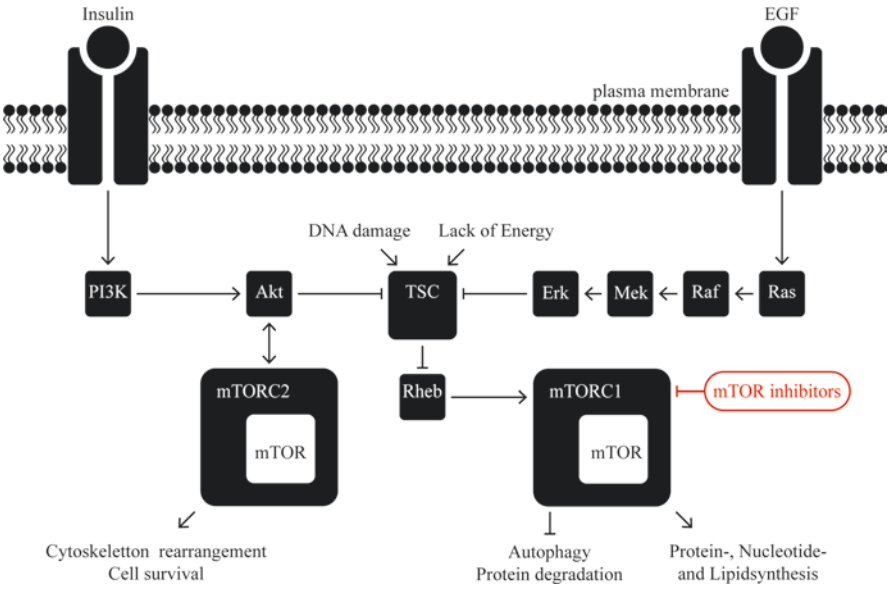

Fig. 16.1 mTOR is the catalytic center of the protein complexes mTORC1 and 2. mTORC1 is activated by the Ras homolog enriched in brain (Rheb) that is in turn inhibited by TSC 1 and 2. TSC is integral to upstream signaling pathways of which cascades responding to DNA damage and energy stress activate TSC. Growth signals, such as the Ras/ERK/MAPK pathway, exemplary stimulated by the epidermal growth factor EGF and the PI3K/Akt pathway initiated by insulin binding to its receptor, inhibit TSC. This results in the activation of mTORC 1 and consequently an anabolic state of the cell by protein, nucleotide, and lipid synthesis, by regulating protein degradation and inhibiting autophagy. mTORC2 is involved in a positive feedback loop involving Akt and has implications on cell survival and cytoskeleton rearrangement. mTOR inhibitors mainly inhibit mTORC1, while mTORC2 is relatively resistant to their effect

The complex effects of mTORC1 and 2 signaling have implications on immune function, the aging process, and the pathogenesis of Alzheimer's disease, diabetes, obesity, and cancer [3]. Whereas the metabolic effects of mTOR-mediated signaling concern all eukaryotic cells, mTOR activity has specific consequences for innate and adaptive immune cells [4]. In T cells, mTORC1 and 2 are activated upon antigen recognition by the T-cell receptor (TCR). mTOR also acts downstream of co-stimulatory molecules and cytokines, signal 2 and 3, that are essential for T-cell activation and proliferation [4]. Inhibition of mTOR activity during antigen presentation was shown to result in T-cell anergy [5] and supports the differentiation of regulatory T cells [6]. In contrast, mTOR inhibition promotes the formation of $CD8^+$ T-cell memory [4]. Due to the largely immunosuppressive effects, the mTOR inhibitor rapamycin, clinically known as sirolimus, was initially approved as an immunosuppressive substance to prevent graft rejection in

kidney transplant recipients in 1999. The drug showed negative effects on the growth of vascular smooth muscle cells and was consequently approved for the coating of coronary artery stents, where it inhibits occlusion. mTOR also plays a critical role in tumorigenesis and in a multitude of cancers mTOR activity is increased [7]. As depicted above, various oncogenic signaling pathways are intertwined with mTORC1 and 2. Exemplary, increased mTOR activity can result from mutations enhancing the Ras/ERK MAPK or PI3K/Akt pathways. Metabolic adaption in cancer cells via mTOR signaling facilitates proliferation and migration and promotes vessel growth in tumors [3]. Hoping to develop a potent anticancer treatment, rapamycin analogues were developed. Compared to sirolimus, everolimus shows increased oral bioavailability, while the prodrug temsirolimus is administered intravenously [8]. Despite the great expectations, these substances showed limited effect in only a few cancer subsets, such as renal cell carcinoma, mantle cell lymphoma, tuberous sclerosis complex patients, and pancreatic neuroendocrine tumors [1]. This is likely explained by the abrogation of the negative feedback loop of mTORC1 on the PI3K/Akt pathway, incomplete inhibition of the phosphorylation of mTORC1 effectors, and relative resistance of mTORC2 to mTOR inhibitors [1]. Scientists are hoping to overcome these limitations by combination of therapies and the development of pan-mTOR inhibitors blocking activity of both mTORC1 and 2 [1]. Temsirolimus is currently approved for the treatment of renal cell carcinoma, and everolimus is used both in posttransplant immunosuppressive regimens and treatment of breast, renal, and neuroendocrine cancers, while another analogue to sirolimus, ridaforolimus, has not reached approval.

Early studies of sirolimus compared to placebo or azathioprine in addition to cyclosporine and corticosteroids in kidney allograft recipients showed no significant difference in the rate of infections, although more mucosal ulcers were observed. On a clinical basis, these mucosal lesions were linked to the herpes simplex virus [9, 10]. Later, impaired wound healing was described in conjunction with sirolimus treatment, leading to a significant increase in perigraft tissue collection and wound infection [11], although the study population was limited in number. Larger randomized trials confirmed the delay in wound healing upon sirolimus administration compared to cyclosporine [12, 13] and tacrolimus [13]. In addition, they revealed a decrease in cytomegalovirus (CMV) infections in the sirolimus group, while high-risk patients (CMV seronegative patients receiving a transplant from a seropositive donor) were equally distributed or even overrepresented in the sirolimus group [12, 13]. Similar results were also reported for everolimus used to prevent kidney graft rejection. A study powered to compare CMV incidence found a 75–90% reduction in patients treated with everolimus versus mycophenolate-based regimens. These patients did not receive antiviral CMV prophylaxis [14]. Both direct antiviral effects of mTOR inhibitors and

changes in CMV immune control have since been suggested to be causative [15]. In a large randomized open-label trial, kidney transplant recipients were stratified regarding the risk of CMV infection (donor and recipient serology) and prophylactic antiviral therapy. Even after adjusting for CMV risk, the rate of infection was significantly lower in the group receiving everolimus versus mycophenolic acid. Interestingly, a reduced rate of BK viruria or viremia was also observed in the everolimus group. Together, this resulted in a lower frequency of all viral infections, while no differences were observed for fungal or bacterial infections [16].

In a meta-analysis of 28 randomized controlled trials, Mallat and colleagues confirmed the lower incidence of CMV infection in mTOR versus calcineurin inhibitor-treated renal transplant patients. The risk ratio was calculated at 0.54, thus almost half the risk of CMV infection [15]. A meta-analysis comparing mTOR inhibitors to mycophenolate or azathioprine came to similar conclusions regarding CMV infections [17]. Due to this anti-CMV effect, switching the immunosuppressive regimen from calcineurin inhibitors to mTOR inhibitors is one strategy suggested to control CMV infections in solid organ-transplanted patients [18]. For BKV infections, however, the meta-analysis mentioned above found no significant difference between mTOR and calcineurin inhibitor-treated patients, likely due to underreporting of the disease in the studies analyzed [15]. While Montero et al. did not report on BKV infections, they compared the discontinuation rates, which were consistently higher in the mTOR inhibitor than the mycophenolate or azathioprine arm. As this highlights tolerability issues connected to mTOR inhibitors, many studies found discontinuation rates to correlate with the dose of mTOR inhibitor administered [17]. While Mallat and colleagues reported no difference in frequency of infections other than BKV and CMV [15], Montero and colleagues saw significant risk reduction for all infections in the first year of mTOR inhibition. The risk in the compared groups, however, equalized after long-term treatment, albeit fewer studies could be included in this analysis [17]. In contrast, an open-label trial, converting immunosuppressive regimens of kidney-transplanted patients form a calcineurin inhibitor to a sirolimus-based treatment, showed more overall infectious adverse events after the switch. Significant differences were observed for pneumonia, stomatitis, presumptive herpes simplex infection, and fever. Such adverse events were particularly frequent in the first 6 months after therapy conversion, while rates equalized between the groups after this time period [19]. The risk of *Pneumocystis jirovecii* pneumonia linked to mTOR inhibition has also been debated in the literature. A

meta-analysis of 15 case-control, cohort studies and randomized controlled studies concluded that mTOR inhibition was associated with an elevated PCP risk. A significant increase of cases was observed after the first year posttransplantation [20], whereas this difference reflects on the net state of immunosuppression or is substance specific remains elusive.

Due to the combination of drugs required to avoid allograft rejection, the study of mTOR inhibitors in this setting involves subjects with a potent therapeutic immunosuppression. In contrast, in patients with neoplastic disorders, the effect of a monotherapy with mTOR inhibitors could be compared to placebo. In addition, tumor patients are generally treated with higher-dose mTOR inhibitors compared to transplant recipients. In randomized phase 3 studies, patients receiving everolimus to treat metastatic renal cell cancer or pancreatic neuroendocrine tumors showed higher rates of infections, stomatitis, and noninfectious pneumonitis [21, 22], of which the latter conditions predispose to infections due to a breakdown of barrier function and can be mistaken for an infection. Three patients with renal cell cancer died due to candida sepsis, presumed bacterial sepsis, or bronchopulmonary aspergillosis [23]. In the population with pancreatic neuroendocrine tumors, a case of tuberculosis, bronchopulmonary aspergillosis, and hepatitis B reactivation was described upon everolimus treatment [22]. A meta-analysis of eight phase 2 and 3 trials treating cancer with either everolimus or temsirolimus yielded an incidence of 33.1% for all-grade infections and 5.6% for serious infections under mTOR inhibition. Comparing mTOR inhibition to the placebo control, a significantly elevated relative risk of 2 and 2.6 was calculated for all-grade infections and high-grade infections, respectively. The relative risks did not significantly differ between the everolimus and the temsirolimus group. Frequently reported infections were localized in the respiratory, genitourinary, and gastrointestinal tract, the skin, and soft tissue or were described as sepsis [24]. A rare genetic disease, called tuberous sclerosis complex, is caused by a mutation in the TSC gene, yields an overactive mTORC1, and results in the formation of benign tumors in multiple organs. In such patients, stomatitis, mouth ulcerations, and pneumonitis were detected more frequently in the everolimus compared to the placebo group [25] consistent with the studies in cancer patients. Rates of respiratory tract infections were particularly high in everolimus-treated patients [25–27]. The frequency of these adverse events decreased during long-term treatment [26]. Table 16.1 lists the trials highlighted above, while relevant infections and action points are summarized in Table 16.2.

Table 16.1 Clinical trials and pooled analyses referenced in Chap. 2

	Year of publication	Author	Trial characteristics	Trial Registration Number	Number of patients	Condition	Substances compared
mTOR inhibitors	2000	Kahan et al.	Randomized, double-blind		719	Renal TPL	Sirolimus vs. azathioprin
	2001	MacDonald et al.	Randomized, double-blind, phase 3		576	Renal TPL	Sirolimus vs. placebo + CNI + corticosteroids
	2004	Dean et al.	Randomized		123	Renal TPL	Sirolimus vs. tacrolimus
	2007	Büchler et al.	Randomized		150	Renal TPL	Sirolimus vs. cyclosporin
	2007	Ekberg et al.	Randomized, open-label	NCT00231764	1645	Renal TPL	Sirolimus vs. cyclosporine vs. tacrolimus
	2009	Schena et al.	Randomized, open-label		830	Renal TPL	Conversion from cyclosporine to sirolimus
	2015	Tedesco-Silva et al.	Randomized, open label	NCT01354301	288	Renal TPL	Everolimus regimen vs. standard treatment
	2019	Tedesco-Silva et al.	Randomized, open-label, 2-arm study	NCT01950819	2026	Renal TPL	Everolimus + CNI + MMF vs. CNI + MMF
	2017	Mallat et al.	Pooled analysis		6211	Renal TPL	Sirolimus/everolimus
	2019	Montero et al.	Pooled analysis		7356	Renal TPL	Sirolimus/everolimus vs. tacrolimus/cyclosporin
	2019	Ghadimi et al.	Pooled analysis		37,597	Solid organ TPL	Sirolimus/everolimus
	2008	Motzer et al.	Randomized, double-blind, phase 3	NCT00410124	410	RCC	Everolimus vs. placebo
	2010	Motzer et al.	Randomized, double-blind, phase 3	NCT00410124	416	RCC	Everolimus vs. placebo
	2011	Yao et al.	Randomized, double-blind, phase 3	NCT00510068	410	Pancreatic neuroendocrine tumours	Everolimus vs. placebo
	2013	Kaymakcalan et.al	Pooled analysis		3180	Varoius cancers	Everolimus/temsirolimus
	2013	Franz et al.	Randomized, double-blind, phase 3	NCT00789828	117	Tuberous sclerosis complex	Everolimus vs. placebo
	2016	Franz et al.	Open-label, single arm	NCT00789828	117	Tuberous sclerosis complex	Everolimus
	2016	French et al.	Randomized, double-blind, phase 3	NCT01713946	366	Tuberous sclerosis complex	Everolimus vs. placebo

JAK inhibitors	2012	Verstovsek et al.	Randomized, double-blind, phase 3	NCT00952289	309	Myelofibrosis	Ruxolitinib vs. placebo
	2012	Harrison et al.	Randomized, open-label, phase 3	NCT00934544	219	Myelofibrosis	Ruxolitinib vs. best available therapy
	2016	Harrison et al.	Randomized, open-label, phase 3	NCT00934544	219	Myelofibrosis	Ruxolitinib vs. best available therapy
	2017	Verstovsek et al.	Randomized, double-blind, phase 3	NCT00952289	299	Myelofibrosis	Ruxolitinib vs. placebo
	2020	Al Ali et al.	Open-label, single arm, phase 3b	NCT01493414	1144	Myelofibrosis	Ruxolitinib
	2020	Zeiser et al.	Randomized, open-label, phase 3	NCT02913261	309	Acute graft-versus-host disease	Ruxolitinib vs. investigators choice
	2015	Vannucchi et al.	Randomized, open-label, phase 3	NCT01243944	222	Polycythemia vera	Ruxolitinib vs. standard treatment
	2020	Kiladjian et al.	Randomized, open-label, phase 3	NCT01243944	222	Polycythemia vera	Ruxolitinib vs. standard treatment
	2012	Fleischmann et al.	Randomized, double-blind, phase 3	NCT00814307	611	Rheumatoid arthritis	Tofacitinib vs. placebo
	2014	Lee et al.	Randomized, double-blind, phase 3	NCT01039688	958	Rheumatoid arthritis	Tofacitinib vs. methotrexate
	2014	Cohen et al.	Pooled analysis		4789	Rheumatoid arthritis	Tofacitinib vs. tofacitinib + MTX or other csDMARDs
	2016	Winthrop et al.	Pooled analysis		5671	Rheumatoid arthritis	Tofacitinib vs. placebo vs. adalimumab vs. MTX
	2017	Winthrop et al.	Pooled analysis		6192	Rheumatoid arthritis	Tofacitinib ± csDMARDs
	2015	Bachelez et al.	Randomized, double-blind, phase 3	NCT01241591	1106	Plaque psoriasis	Tofacitinib vs. etanercept vs. placebo
	2017	Mease et al.	Randomized, double-blind, phase 3	NCT01877668	422	Psoriatic arthritis	Tofacitinib vs. placebo vs. adalimumab
	2017	Sandborn et al.	3 randomized, double-blind, phase 3		1732	Ulcerative colitis	Tofacitinib vs. placebo
	2018	Winthrop et al.	Pooled analysis		1157	Ulcerative colitis	Tofacitinib
	2012	Vincenti et al.	Randomized, phase 2	NCT00483756	331	Renal TPL	Tofacitinib vs. cyclosporine
	2016	Genovese et al.	Randomized, double-blind, phase 3	NCT01721044	527	Rheumatoid arthritis	Baricitinib vs. placebo
	2017	Dougados et al.	Randomized, double-blind, phase 3	NCT01721057	684	Rheumatoid arthritis	Baricitinib vs. placebo
	2017	Taylor et al.	Randomized, double-blind, phase 3	NCT01710358	1307	Rheumatoid arthritis	Baracitinib vs. placebo vs. adalimumab
	2019	Smolen et al.	Pooled analysis		3492	Rheumatoid arthritis	Baricitinib vs. placebo
	2019	Genovese et al.	Randomized, double-blind, phase 3	NCT02873936	448	Rheumatoid arthritis	Filgotinib vs. placebo
	2018	Burmester et al.	Randomized, double-blind, phase 3	NCT02675426	661	Rheumatoid arthritis	Upadacitinib vs. placebo
	2019	Fleischmann et al.	Randomized, double-blind, phase 3	NCT02629159	1629	Rheumatoid arthritis	Upadacitinib vs. placebo vs. adalimumab + MTX
	2019	Tanaka et al.	Randomized, double-blind, phase 3	NCT02308163	507	Rheumatoid arthritis	Peficitinib vs. placebo vs. etanercept

(continued)

Table 16.1 (continued)

	Year of publication	Author	Trial characteristics	Trial Registration Number	Number of patients	Condition	Substances compared
BCL-2 inhibitors	2016	Roberts et al.	Open-label, phase 1	NCT01328626	116	Relapsed or refractory CLL or SLL	Venetoclax
	2018	Seymour et al.	Randomized, open-label, phase 3	NCT02005471	389	Relapsed/refractory CLL	Venetoclax + rituximab vs. bendamustine + rituximab
	2018	Stilgenbauer et al.	Open-label, phase 2	NCT01889187	158	Relapsed/refractory CLL with 17p deletion	Venetoclax
	2019	Fisher et al.	Randomized, open-label, phase 3	NCT02242942	432	Previously untreated CLL	Venetoclax + obinutuzumab vs. chlorambucil + obinutuzumab
	2020	DiNardo et al.	Randomized, double-blind, phase 3	NCT02993523	431	Previously untreated AML	Venetoclax + azacitidine vs. + placebo + azacitidine

MTX methotrexate, *csDMARDs* conventional synthetic disease-modifying anti-rheumatic drugs, *TPL* transplantation, *CNI* calcineurin inhibitor, *MMF* mycophenolate, *RCC* renal cell carcinoma, *CLL* chronic lymphatic leukaemia, *SLL* small lymphocytic lymphoma, *AML* acute myeloid leukaemia

Table 16.2 Infectious risk connected with mTOR, JAK, and BCL-2 inhibiting substances

Substance	Infection/condition	Risk	Suggested management
mTOR inhibitors	Overall viral infections	Elevated	Yearly influenza vaccination
	Herpes simplex/zoster	Elevated	Prophylaxis in subjects with additional risk (e.g. after transplantation)
	CMV	Decreased	
	BKV	Possibly decreased	
	HBV	Elevated	Screening for HBV
			Treatment in patients with detectable HBV DNA and/or HBs-ag
			HBV DNA monitoring in patients with undetectable HBV DNA and/or HBs-ag but positive HBc-antibody
	Overall bacterial infections	Elevated	Consider prophylaxis in subjects with additional risk (e.g. neutropenic patients)
	Tuberculosis	Elevated	Screening for latent tuberculosis in patients from non-endemic regions
			Treatment of latent tuberculosis upon positive screening or in patients form endemic regions
	Fungal infections	Rare cases	Consider prophylaxis in subjects with additional risk (e.g. neutropenic patients)
	Pneumocystis jirovecii pneumonia	Possibly elevated	Prophylaxis in subjects with additional risk (e.g. upon corticosteroid use, after transplantation)
	Impaired wound healing, mucositis, pneumonitis	Elevated	Risk of superinfection

(continued)

Table 16.2 (continued)

Substance	Infection/condition	Risk	Suggested management
JAK inhibitors	Overall viral infections	Elevated	Yearly influenza vaccination
	Herpes zoster	Elevated	Screening for VZV IgG
			VZV or HZ vaccination before treatment
			Prophylaxis in subjects with additional risk (e.g. after transplantation)
	CMV	Elevated in presence of potent immunosuppression	Prophylaxis in subjects with additional risk (e.g. after transplantation)
	BKV, EBV	Possibly elevated in presence of potent immunosuppression	Monitoring
	HBV	Elevated	Screening for HBV
			Treatment in patients with detectable HBV DNA and/or HBs-ag
			HBV DNA monitoring in patients with undetectable HBV DNA and/or HBs-ag but positive HBc-antibody
	Overall bacterial infections	Elevated	Consider prophylaxis in subjects with additional risk (e.g. neutropenic patients)
	Tuberculosis	Elevated	Screening for latent tuberculosis in patients from non-endemic regions
			Treatment of latent tuberculosis upon positive screening or in patients form endemic regions
	Fungal infections	Rare cases	Consider prophylaxis in subjects with additional risk (e.g. neutropenic patients)
	Pneumocystis jirovecii pneumonia	Elevated	Prophylaxis in subjects with additional risk (e.g. upon corticosteroid use, after transplantation)
BCL-2 inhibitors	Neutropenia	Elevated	Risk for neutropenic fever

VZV varicella zoster virus, *HZ* herpes zoster, *CMV* cytomegalovirus, *BKV* BK virus, *EBV* Epstein-Barr-virus, *HBV* hepatitis B virus

JAK Inhibitors

Janus kinases (JAKs) are involved in the intracellular signal transduction downstream of cytokine, colony-stimulating factor, growth factor, and hormone receptors (Fig. 16.2). Such receptors are essential for hematopoiesis, metabolism, and immunity [28]. Upon interaction with the respective ligand, the receptors oligomerize, bringing JAKs, non-covalently bound to the cytoplasmic domain of the receptor, into close proximity. This leads to the phosphorylation of JAKs, the cytokine receptors, and target molecules such as signal transducers and activators of transcription (STAT). STAT, upon activation, dimerize and translocate into the nucleus where they regulate transcription of a variety of genes [29]. The JAK-STAT pathway was first discovered in conjunction with interferon signaling [30], highlighting its importance in the pathogenesis of autoimmunity, infection, and cancer. There are four mammalian members of the JAK family—JAK 1, JAK 2, JAK 3, and TYK2—involved in the signaling of many more receptors [28]. JAK 3 exemplary binds to the common γ chain (γc) of cytokine receptors and hereby is essential for signaling downstream of interleukin (IL) -2, −4, −7, −9, −15, and −21 [28]. Defective signal

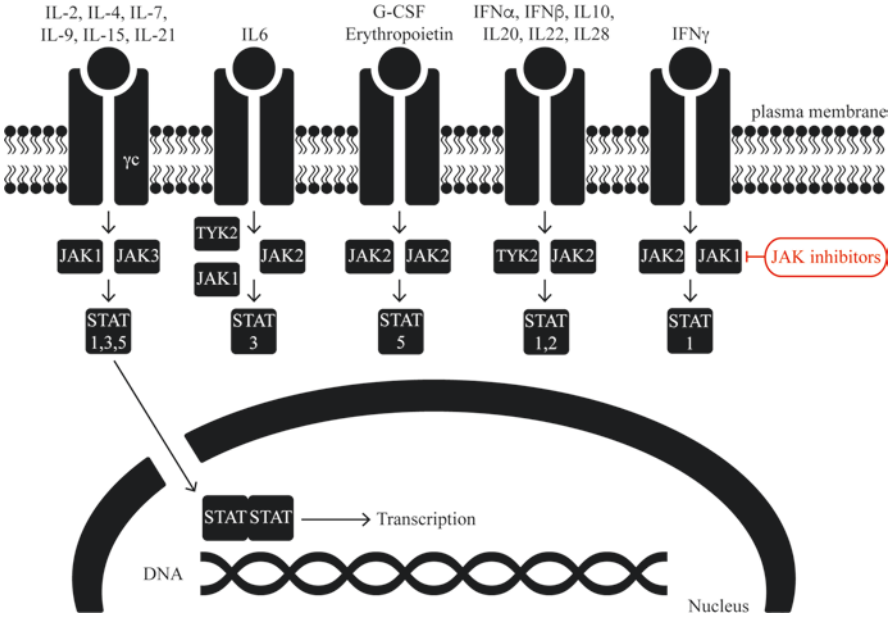

Fig. 16.2 The four members of the JAK family (JAK1, 2, 3, and Tyk2) are activated downstream of growth factors (e.g., G-CSF and erythropoietin) and cytokines (various interleukins (IL) and interferons (IFN) binding to their corresponding receptor. Among other proteins, STATs are phosphorylated by JAKs, dimerize, and induce transcription in the nucleus. Different receptors will engage a distinct set of JAKs, which will further determine the STAT protein activated and the genes targeted. JAK inhibitors inhibit one or multiple JAK family members. JAK inhibitors applied for hematological malignancies aim for JAK2 downstream of growth receptor signaling, while in autoimmune disease, JAK1 and 3, involved in cytokine signaling, are targeted

transduction, caused by JAK 3 loss of function mutations, leads to severe combined immunodeficiency (SCID) in mice and humans, illustrating the nonredundant role of JAK 3 for immune function [28]. Human SCID is commonly the result of the X chromosome-linked mutation of the γc gene [31]. JAK 3 mutations result in the same phenotype due to the deficient development, homeostasis, and activation of T and NK cells [28]. TYK 2 dysfunction due to germ line mutations has rarely been described in humans and seems to vary regarding the phenotypic presentation of immunodeficiency syndromes [28]. JAK 1 and 2 associate with a larger variety of receptors involved in immune signaling, hematopoiesis, growth, and organ development. Just as for JAK 3, JAK 1 activity is required for the signal transduction downstream of γc cytokine receptors but additionally associated with receptors of the IL-6 family cytokines, IL-10, IL-13, and IL-22, type 1 and 2 interferons (IFN) and GCSF. JAK 2 is essential for signaling via the IL-6 and IL-3 family receptors, IL-12, IL-23, IL-13, and INFγ and receptors involved in hematopoiesis (e.g., erythropoietin, GCSF) [28]. Genetic knockout of both JAK 1 and 2 in mice results in a lethal phenotype [28, 29], which is why there is no human correlate for disease. In contrast, a gain of function mutation in JAK genes, particularly for JAK 2, can result in neoplastic growth, primarily of hematopoietic origin [28]. JAK 2 has been implicated in the pathogenesis of leukemia, lymphoma, thrombocytemia, and particularly in polycythemia vera and myelofibrosis. Increased JAK 1 and 3 activity is reported in the development of monoclonal malignancies of hematological origin [28].

JAK activation plays a role in the pathogenesis of inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis [32]. Polymorphisms in JAK2-STAT3 have been implicated in different inflammatory conditions [28].

Several JAK inhibiting substances have been approved, and many more are in clinical trials, each compound targeting different combinations of JAKs. The first generation of JAK inhibitors (JAKinibs) are broader in their specificity and inhibit the activation of multiple JAKs [33]. In the attempt to avoid side effects later, substances were developed to more specifically bind to one JAK. Despite these improvements, all JAKinibs inhibit other JAK family members when applied at high dose, thus displaying similar adverse effects [32].

The JAK1-JAK2 inhibitor ruxolitinib was the first substance being evaluated for the treatment of myelofibrosis in clinical trials [28]. It has since been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of myelofibrosis and polycythemia vera. Two randomized clinical trials comparing ruxolitinib to placebo [34] or the best available treatment [35] did not suggest an added risk for infection under ruxolitinib. The long-term study of the patient population, however, revealed a link between ruxolitinib treatment and herpes zoster [36, 37]. Similar results were obtained in a phase 3 open-label study, in which polycythemia vera patients resistant or intolerant to hydroxyurea treatment were observed. Herpes zoster, mostly grade 1 or 2, was reported in 6% versus 0% in patients given ruxolitinib or standard care, respectively [38]. The 5-year follow-up study confirmed the link of ruxolitinib with herpes zoster, as the

patients who crossed over from best available treatment to the study drug approached herpes zoster rates of the ruxolitinib group (3.9% vs. 4.7%). With the exception of herpes zoster, the study showed a reduction in all infections in ruxolitinib-treated patients compared to control [39]. A large single-arm, open-label, phase 3b study, in patients with myelofibrosis under ruxolitinib treatment, showed low rates of infections [40]. These mostly low-grade infections involved pneumonia, urinary tract infections, herpes zoster, and nasopharyngitis. They observed five cases of tuberculosis, one hepatitis B reactivation, but no patient developed progressive multifocal leukencephalopathy [40]. In patients with acute corticosteroid refractory graft versus host disease (GVHD), ruxolitinib was compared to the investigator's choice of salvage therapy, and more CMV infections were observed in the ruxolitinib group compared to control (26% vs. 21%). This difference was not demonstrated for grade 3 and 4 infections [41].

The majority of data concerning the safety of JAKinibs are derived from studies of tofacitinib, a JAK 1/3 inhibitor. It is now approved for the treatment of rheumatoid arthritis, psoriasis arthritis, and ulcerative colitis. A phase 3 trial in rheumatoid arthritis described an increased rate of serious infections under the tofacitinib compared to placebo. The infections involved skin (including one case of herpes zoster), respiratory tract, urinary tract, and liver [42]. Higher rates of all-grade infections and serious infections were also reported in tofacitinib groups compared to placebo in ulcerative colitis patients [43]. In rheumatoid arthritis patients, herpes zoster infections developed in 4% of the tofacitinib versus 1.1% of the methotrexate-treated subjects. A dose-dependent effect was suggested as the group receiving 5 mg tofacitinib developed herpes zoster in 3.5%, compared to 4.5% in the 10 mg group. Bronchitis and influenza were also observed more frequently in tofacitinib- versus methotrexate-treated patients [44]. In psoriasis patients, two phase 3 trials comparing tofacitinib, etanercept, and placebo [45] or tofacitinib, adalimumab, and placebo [46], the treated groups showed similar rates of adverse events [45, 46]. Over the study period, only a few patients experienced serious infections including diverticulitis, an extradural abscess, pneumonia, and paronychia [45], and influenza, appendicitis, and pneumonia [46] in the tofacitinib groups. Whereas Bachelez et al. did not see a difference in herpes zoster rates between the treated groups, Mease et al. only observed herpes zoster infection upon tofacitinib treatment. A pooled analysis investigated rates of infections in phase 2/3 and long-term extension studies treating rheumatoid arthritis patients with tofacitinib [47]. With 3.09 events per 100 patient-years upon treatment, tofacitinib was comparable to other biologic agents regarding serious infections. The most common serious infections were pneumonia and infections of skin or soft tissue. The majority of infections were, however, moderate in severity, and exposure-adjusted event rates in the phase 3 studies were comparable between tofacitinib- and placebo-treated groups. Consistent with previous results, tofacitinib treatment was linked to herpes zoster infections. While most cases were mild, four cases of zoster ophthalmicus and two cases of multi-dermatomal disease were described. There were three cases of HBV infection with one accounting for a possible reactivation. 41 opportunistic infections were reported including patients suffering from tuberculosis, esophageal candidiasis,

CMV infection, cryptococcal infection, *Pneumocystis jirovecii* pneumonia, nontuberculous mycobacteria infections, multi-dermatomal herpes zoster, and BKV encephalitis [47]. Winthrop et al. aimed at characterizing severity, geographical distribution, and the role of concomitant therapy of herpes zoster infections in patients treated with tofacitinib for rheumatoid arthritis from phase 1 to 3 and long-term studies. In 6192 patients (16,839 patient-years), 636 cases of herpes zoster were identified of which 94% were involving one dermatome, and disease was generally manageable with antiviral treatment. Concomitant corticosteroid administration, baseline age, and dose of daily tofacitinib were independent risk factors [48]. Similar results were observed in ulcerative colitis patients, although lower patient numbers only allowed identifying age and prior failure of TNF inhibitors as independent risk factors [49]. Herpes zoster was observed more frequently in east-Asian countries, implying underlying genetic differences to be causative, and gene polymorphisms have been suggested [48]. Both studies showed no evidence for a herpes zoster-related risk accumulation over exposure time [48, 49]. In a phase 2 clinical trial of de novo kidney-transplanted patients, tofacitinib was compared to cyclosporine in addition to basiliximab induction mycophenolic acid and corticosteroids [50]. Overall, serious infections were observed more frequently in the tofacitinib group. While there was no difference between the groups for upper airway and urinary tract infections, there was significantly more CMV disease under tofacitinib. Although low in numbers, BKV nephropathy and PTLD developed more often in tofacitinib-treated patients, reflecting on the potential over-immunosuppression [50]. In a pooled analysis of phase 2, 3, and long-term extension clinical trials of tofacitinib-treated rheumatoid arthritis cases, such infections related to immunosuppression were investigated [51]. In a population of 5671 patients, 60 opportunistic infections were described, all within the tofacitinib-treated group. These encompassed cases of tuberculosis, esophageal candidiasis, disseminated herpes zoster manifestations, CMV infections, *Pneumocystis jirovecii* pneumonias, nontuberculous mycobacteria infections, cryptococcal diseases, BK encephalitis, and toxoplasmosis. Among 286 patients with a positive screening for latent tuberculosis, and consequent 9-month isoniazid treatment, no case of active tuberculosis was observed. Out of the 26 subjects developing tuberculosis, 24 had negative screenings at inclusion. Of the tuberculosis cases, 81% emerged in endemic regions [51], although no subjects lived in countries with the highest incidence according to the WHO.

Another reversible JAK1/3 inhibitor, baricitinib, showed very low frequencies of serious infections and herpes zoster in placebo-controlled studies of patients with rheumatoid arthritis [52, 53]. Similar to other JAKinibs, respiratory infections [52, 53] and urinary tract infections [53] were among the most frequent adverse events. A comparison of baricitinib and adalimumab showed similar rates of serious infections and herpes zoster [54]. A pooled analysis of phase 1–3 and long-term studies showed an increase in infections only in patients treated with high-dose baricitinib (4 mg) compared to placebo. Higher-exposure-adjusted incidence rates were shown for upper respiratory tract infections, herpes zoster, and herpes simplex. Serious infections, such as pneumonia herpes zoster, urinary tract infections, and cellulitis

were the most common in the baricitinib group, but incidence rates were similar in patients receiving placebo. Under baricitinib, ten patients developed tuberculosis [55]. With a lack of head-to-head analysis, it remains unclear whether incidences of specific infections differ between tofacitinib and baricitinib.

Similar to others, the pan-JAKinibs peficitinib treatment was connected to an increased incidence of serious infections compared to placebo in rheumatoid arthritis, but comparable to patients under etanercept treatment. It is only for herpes zoster infections that peficitinib showed a higher incidence than both the placebo and etanercept group [56]. Peficitinib is approved in Japan for the treatment of rheumatoid arthritis.

A higher incidence of infections was reported in rheumatoid arthritis patients treated with the specific JAK 1 inhibitor upadacitinib compared to placebo, although no difference was observed for serious infections opportunistic infections or herpes zoster [57]. The serious infections in the upadacitinib groups involved one case for each enterocolitis, upper respiratory tract infection, wound infection, and a primary varicella zoster infection leading to VZV pneumonia. The high-dose upadacitinib group included three patients with oral candidiasis [57]. A phase 3 trial comparing upadacitinib with placebo and adalimumab on a methotrexate background treatment saw similar frequencies of infections and serious infections for both treatment groups. Herpes zoster was the only infection with higher rates in the upadacitinib compared to the placebo and adalimumab group [58]. Similar results were shown for filgotinib, another JAK 1 inhibitor, as more infections were observed in the treatment versus the placebo group in rheumatoid arthritis patients. Herpes zoster, although only few cases, was only observed in the filgotinib groups [59]. Table 16.1 lists the trials highlighted above, while relevant infections and action points are summarized in Table 16.2.

BCL-2 Inhibitors

B-cell lymphoma 2 (BCL-2) family proteins are involved in the regulation of the mitochondrial pathway of apoptosis (Fig. 16.3). They share a combination of one to four conserved BCL-2 homology (BH) domains, which determine their anti- or proapoptotic function [60]. The interplay of the BCL-2 family members creates a balance between cell survival and death, which can be shifted by physiological signals and pathological dysregulation of the proteins involved. Within the family, the antiapoptotic BCL-2 was discovered first in follicular lymphomas and diffuse large B-cell lymphomas. In such tumors, a chromosomal translocation results in cancer cell survival through a BCL-2 gain of function. Since this discovery, other antiapoptotic BCL-2 family members, such as B-cell lymphoma extra large (BCL-X_L), BCL-W, and myeloid cell leukemia 1 (MCL1), have been characterized. Such antiapoptotic BCL-2 proteins counteract the proapoptotic function of BCL-2 antagonist killer 1 (BAK) and BCL-2-associated X protein (BAX). This, in turn, prevents an increased permeability of the mitochondrial membrane, the release of cytochrome C into the cytoplasm, and subsequent activation of the caspase cascade resulting in

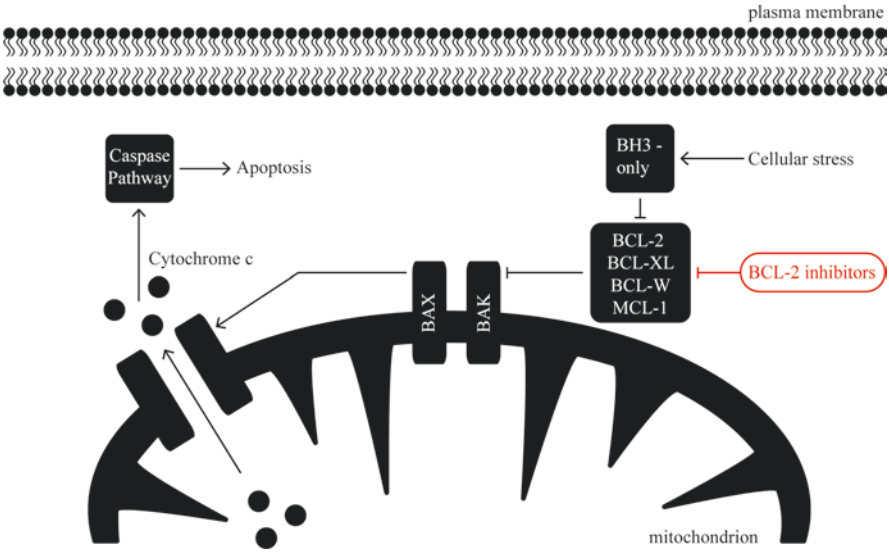

Fig. 16.3 Pro-survival BCL-2 family members, such as BCL-2, BCL-XL, BCL-W, and MCL-1, inhibit their proapoptotic counterparts BAK and BAX. This in turn inhibits the permeabilization of the mitochondrial membrane, the release of cytochrome c into the cytoplasm, and subsequently prevents apoptosis by the activation of the caspase cascade. Pro-survival BCL-2 proteins are inhibited by BH3-only proteins, which are activated by cellular stress. BCL-2 inhibitors mimic the function of BH3-only proteins, promoting apoptosis and thereby counteracting deregulated survival signals in cancer cells. While early BCL-2 inhibitors affected multiple pro-survival BCL-2 proteins leading to adverse thrombocytopenia, venetoclax specifically inhibits BCL-2, avoiding this adverse event

apoptosis [60]. Upstream, pro-survival BCL-2 proteins are inhibited by BCL-2 family members only containing the BH3 domain, therefore referred to as BH3-only proteins. Apart from this indirect induction of apoptosis, some BH3-only proteins can directly interact with BAK and BAX [61]. BH3-only proteins are activated by intracellular stress signals, such as DNA damage, oxidative stress, or the lack of growth factor signaling. Exemplary, in response to DNA damage, the tumor suppressor p53 induces transcription of certain BH3-only proteins. A mutation resulting in p53 loss of function is observed in as many as 50% of cancers [62]. Due to this frequent dysregulation of BCL-2 activity in malignancies, pharmacological substances aiming to inhibit BCL-2 function were developed. Such small molecules were termed BH3 mimetics, as they reproduce the mechanism by which BH3-only proteins inhibit pro-survival BCL-2 signaling [63]. The first promising BH3-mimetic was termed ABT-737 and was followed by navitoclax, a substance with improved oral bioavailability compared to its predecessor. Both drugs mainly inhibit BCL-2, BCL-X_L, and BCL-W. Despite antitumor efficacy in phase 1 trials, navitoclax never reached approval due to its negative impact on thrombocyte survival, an on-target effect involving inhibition of BCL-X_L function [63]. To avoid this undesirable effect, more recent BH3-mimetics were designed to show higher specificity.

The BCL-2-selective inhibitor venetoclax provoked a solid antitumor response, while thrombocytopenia was less severe [64]. Venetoclax is the first BH3-mimetic approved by the FDA and the EMA for patients with chronic lymphatic leukemia [63]. More recently, the combination therapy with several anticancer substances has reached approval. Moreover, venetoclax is being studied in various other cancer types. While no other drugs interfering with BCL-2 protein-associated apoptosis are in clinical use, the development MCL-1 inhibitors is of great interest, and several drugs are in clinical trials.

Due to the recent approval for venetoclax, up to now, there is a limited amount of articles studying safety. In a dose-escalation phase 1 trial of CLL and small lymphocytic lymphoma, patients that relapsed after or proved refractory to initial treatment were administered with venetoclax. Neutropenia was a frequent adverse event, in some cases progressing to episodes of febrile neutropenia. Upper respiratory tract infections and pneumonia were other infections reported [65]. Similar observations were made in a phase 2 study in CLL patients with a genetic 17p deletion, a finding related to poor prognosis [66]. In addition to the febrile neutropenia and respiratory infections, this trial reported cutaneous herpes zoster and *Pneumocystis jirovecii* pneumonia, although the latter was only observed in patients previously treated with the chemotherapeutic fludarabine. Four patients succumb to a RSV infection, *Klebsiella* sepsis, septic shock, and pneumonia [66]. In a randomized, double-blind phase 3 trial in AML patients, venetoclax was compared to placebo, while all patients received azacitidine, a hypomethylating agent. Neutropenia, febrile neutropenia, and all-grade infections were observed more frequently in the venetoclax group, while there was no difference in rates of pneumonia and sepsis compared to control [67]. In most studies, venetoclax was investigated in comparison to other antitumor therapeutics frequently used to treat CLL patients. Receiving a combination of rituximab (an anti-CD20 antibody) with either venetoclax or the alkylating agent bendamustine, grade 3/4 neutropenia was more common in the venetoclax group, while grade 3/4 febrile neutropenia and infections were more frequent under bendamustine treatment [68]. Comparing venetoclax with the alkylating agent chlorambucil, both in combination with the anti-CD20 antibody obinutuzumab accounted for similar rates of neutropenia, febrile neutropenia, and infections [69]. Table 16.1 lists the trials highlighted above, while relevant infections and action points are summarized in Table 16.2.

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