REVIEW ARTICLE

Kinases as potential targets for treatment of pulmonary hypertension and right ventricular dysfunction

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Pulmonary hypertension (PH) is a progressive pulmonary vasculopathy that causes chronic right ventricular pressure overload and often leads to right ventricular failure. Various kinase inhibitors have been studied in the setting of PH and either improved or worsened the disease, highlighting the importance of understanding the specific role of the respective kinases in a spatiotemporal cellular context. In this review, we will summarize the knowledge on the role of kinases in PH and focus on druggable targets for which certain criteria are met: (a) deregulation of the kinase in PH; (b) small-molecule inhibitors are available (e.g. from the oncology field); (c) preclinical studies have shown their efficacy in PH models; and (d) when available, therapeutic exploitation in human PH has been initiated. Along this line, clinical considerations such as personalized medicine approaches to predict therapy response and adverse side events such as cardiotoxicity together with their clinical management are discussed.

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Abbreviations:
4E-BP1, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; 6-MWD, 6-min walking distance; Abl, Abelson protooncogene; ALK, activin receptor-like kinases; Ask1, apoptosis signal-regulating kinase 1; Bcr-Abl, breakpoint cluster region-Abelson protooncogene (fusion gene product); BID, bid, bax, bid in die, twice a day; BMP, bone morphogenetic protein; BMPR2, bone morphogenetic protein receptor 2; Cap, density, capillary density; CDK, cyclin-dependent kinases; CHF, chronic heart failure; c-KIT, CD117, stem cell factor receptor; CM, cardiomyocyte; CML, chronic myeloid leukemia; COPD, chronic obstructive lung disease; CSF1R, colony stimulating factor 1 receptor; DCA, dichloroacetate; DDR, discoidin domain receptor; EGFR, EGF receptor; eIF2E, eukaryotic initiation factor 2E; ER, endoplasmic reticulum; FDA, Food and Drug Administration; FGFR, fibroblast-derived growth factor receptor; FK506, tacrolimus; FKB12, 12-kDa FK506-binding protein; FLT3, Fms like TK 3; Fos, forkhead box O; GDF, growth and differentiation factors; GIST, gastrointestinal stromal tumours; GSK, GlaxoSmithKline; Glit, piramidinum; gR, target, receptor guide; HGF, heart failure with preserved ejection fraction; Id, inhibitor of DNA binding; IGF-1R, insulin-like growth factor receptor; IMPRES, imatinib in PAH, a randomized efficacy study; IPAH, idiopathic pulmonary arterial hypertension; iPF, idiopathic fibrosis; L, left ventricle; LVD, left ventricular dysfunction; LV, left ventricular ejection fraction; MCT, monocrotaline; MI, myocardial infarction; MLCK, myosin light chain kinase; mPAP, mean pulmonary arterial pressure; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; nd., not determined; NFAT, nuclear factor of activated T-cells; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PA, pulmonary artery; PAB, pulmonary artery banding; PAECs, pulmonary artery endothelial cells; PAH, pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PDH, pyruvate dehydrogenase complex; PD-1, programmed death ligand 1; PH, pulmonary hypertension; PI3K, phosphoinositide 3-kinases; PMLC, phospholipase C gamma; PPIs, protein serine/threonine phosphatases; PTPs, protein tyrosine phosphatases; PVR, pulmonary vascular resistance; Rb, retinoblastoma protein; ROCK, Rho-kinase; RV, right ventricle; RVH, right ventricular hypertrophy; RVSP, right ventricular systolic pressure; S1P, sphingosine-1-phosphate; S6K1, p70 ribosomal protein S6 kinase 1; S6K1, p70 S6 kinase 1; SAE, serious adverse effects; SFH-1, soluble Fms like TK 1; Shp2, Src homology 2 containing protein tyrosine phosphatase 2; TAK1, transforming growth factor beta; TGF-J, TGF-J receptor; TKI, TK inhibitor; UCP2, uncoupling protein 2; VEGF, vascular endothelial cell-derived growth factor receptor.
1 | KINASE SIGNALLING AND INHIBITION IN PULMONARY HYPERTENSION

The pathobiological key finding in pulmonary hypertension (PH) regardless of the different aetiologies is the structural remodelling process within the pulmonary vasculature. This remodelling involves several cell types including pulmonary arterial smooth muscle (PASMCs) and endothelial cells (PAECs) and adventitial fibroblasts as well as cells from the immune system. A complex phenotype of increased proliferation, vasoconstriction, and apoptosis resistance, together with inflammation and in situ thrombosis, can be considered as the common disease origin (Guignabert et al., 2013; Schermuly, Ghofrani, Wilkins, & Grimminger, 2011; Tudor et al., 2013). The luminal obstruction leads to an increase in pulmonary artery pressure, right ventricular hypertrophy, and, if untreated, death due to right heart failure. The ability of the right ventricle (RV) to compensate for changes in load has important prognostic value in PAH (Tello et al., 2019; van de Veerdonk et al., 2011) and RV failure due to pressure overload remains the predominant cause of death (Benza et al., 2012). Beside vasoactive mediators like NO, endothelin or prostacyclin and growth factors like PDGF, FGF or EGF are critically involved in the remodelling process. As reviewed elsewhere, growth factors signal via their respective receptors (Alexander, Fabbro, et al., 2017a) and initiate kinase-dependent signalling events ultimately leading to increased proliferation, resistance to apoptosis and metabolic changes. The group of kinases consists of 518 members which phosphorylate their substrates either on tyrosine (TK) or on serine/threonine (serine/threonine kinase) residues. Kinases can also be classified due to their cellular localization, trans-membranous or intracellular. They all serve as master regulators of cellular fate including virtually all signalling pathways from cell regeneration to cell death. In the past, several kinases have been described to be crucial for the altered cellular phenotype and the dysfunction of pathways which not only regulate vascular tone and blood flow in the pulmonary circulation but also control the growth of the pulmonary vascular cells. For some of them, an aberrant activity has also been shown to be responsible for tumour development. Kinase inhibitors already approved in the oncology field can be of great value for the treatment of PH (reviewed in Woodcock & Chan, 2019).

2 | EFFICACY AND SAFETY OF KINASE INHIBITOR THERAPY IN PULMONARY HYPERTENSION

The increase in activity of a kinase under disease conditions as compared with control situations is one important parameter that dictates the side-effect profile of a potential drug. This aspect is of great importance with regard to the risk of toxicity observed for cardiac function and mitochondrial dysfunction as this hampers clinical safety of kinase inhibitors. A few inhibitors have been reported in cancer trials to trigger cardiotoxicity with adverse cardiac events and increased heart failure hospitalizations (Chu et al., 2007; Kerkela et al., 2006). While this emphasizes the need for routine cardiac monitoring in cancer trials (Chaar, Kamta, & Ait-Oudhia, 2018), kinase inhibitor cardiotoxicity is not a drug class effect, and the expression level of certain kinases in cardiomyocytes does not necessarily correlate with the toxicity induced by their corresponding inhibitor (Orphanos, Ioannidis, & Ardavanis, 2009). It is the cellular function of the respective kinase that is the major determining factor of whether inhibition of this kinase exerts cardiotoxicity. Therefore, it is crucial to carefully examine the direct effects of kinase inhibitors on the myocardium when re-purposing kinase inhibitors developed as cancer treatments. Along the same line, kinase inhibitors need to be studied under stress conditions as unwanted cardiotoxic effects may be hidden in the healthy myocardium and may be related to ischaemia-related cell death (hidden cardiotoxicity) (Ferdinandy et al., 2019). Further, kinase inhibitors often affect the whole cardio-pulmonary system, and therefore, adverse cardiac events are potentially masked by beneficial anti-remodelling effects in the lung vasculature in preclinical PH settings. These considerations underline the need for an in-depth characterization of a particular kinase in the cardio-pulmonary unit, especially since unexpected adverse cardiac effects are the leading cause of discontinuation of clinical trials and withdrawal of drugs from the market. Therefore, best practices require several experimental PH models in different animal species to concisely describe the role for a particular kinase in the lung and heart (Bonn et al., 2017).

Direct effects of pharmacological compounds on RV structure and function in the context of PH are best studied in models with an increased but fixed afterload, where direct effects on the RV myocardium are studied without interfering afterload alterations due to lung remodelling. This is, for instance, achieved through surgical banding of the main pulmonary artery (PA) to a defined diameter (Maaran, Lecour, Butrous, Thienemann, & Sliwa, 2013). Depending on the degree of PA stenosis and duration of maintained pressure overload, these animals either develop RV dysfunction with compensated RV hypertrophy or RV failure (Egemenazar, Crnkovic, Nagy, Olszewski, & Kwapiszewksa, 2018), very similar to what is seen in PAH patients (Tello et al., 2019). By utilizing this animal model, direct cardiotoxic effects of kinase inhibitor treatment and their effects hidden in the healthy heart (hidden cardiotoxicity (Ferdinandy et al., 2019) can be unraveled. This is important as those cardiovascular adverse effects are the main factor complicating the use of kinase inhibitors in humans. Cardiovascular-related adverse effects include hypertension, QT interval prolongation, a potentially life-threatening abnormality of cardiac repolarization, or left ventricular dysfunction (Dong & Chen, 2018). Further, cardiovascular adverse effects can vary from non-symptomatic decrease in ejection fraction or increase in natriuretic peptides to life-threatening heart failure or pressure increase (Lenihan & Kowey, 2013). Cardiovascular adverse effects seem to be drug specific. Therefore, the use of kinase inhibitors only succeeds under careful monitoring for cardiotoxicity signs. Monitoring for cardiotoxicity should include regular echocardiographic evaluation of cardiac function, ECG with special attention to QT interval prolongation and monitoring of cardiac biomarkers such as creatinine kinase,
troponin, and natriuretic peptides (Schmidinger et al., 2008). No recommendations exist about the frequency of the monitoring, but for oncological patients, the frequency of monitoring has been increased up to bimonthly.

Finally, although there is a high similarity between cancer cells and pulmonary vascular cells with respect to the hyper-proliferative phenotype, it seems to be more complex to simply adapt the same kinase inhibitors to PH. In solid tumours as well as in PH, several cell types interact within their distinct network together to establish, maintain, and to expand the disease associated cellular composition and structure while there are still enough differences between those two diseases: Tumour cells are needed neither for the proper function of the affected organ nor for the survival of the organism. They possess an aberrant phenotype which can be clearly distinguished from the initial status of their healthy counterparts. This is opposite in the case of PH; although diseased cells of the lung or the heart develop an abnormal phenotype with the given characteristic in PH (e.g., hyper-proliferation, resistance to apoptosis, hypertrophy, and fibrosis), they are still embedded in their organ structure and need to be functional in this cellular context unless they can be replaced by normal pulmonary vascular or cardiac cells. For this reason, therapeutic strategies targeting kinase centrally involved in crucial signalling pathways (i.e., survival, apoptosis, hypertrophy, and fibrosis) have to be chosen carefully to restore and maintain functional integrity.

3 | GROWTH FACTOR RECEPTOR SIGNALLING

One of the first kinases identified to be crucial for development of PH is PDGFRβ (Schermuly et al., 2005). It is primarily expressed on PASMCs and activated by its ligand (PDGF) to induce major signalling pathways like Ras/MAPK, PI3K, and PLCγ, leading to transcriptional programs with an increase in expression of genes responsible for proliferation, resistance to apoptosis and migration. Since 2001, imatinib, a TK inhibitor, is an approved drug for the treatment of CML (chronic myeloid leukaemia) and GIST (gastrointestinal stromal tumours; since 2002) targeting PDGFRβ and two other kinases involved in cancer development, namely, c-Kit and Abl. Ghofrani et al. have reported successful treatment of a PAH patient refractory to standard therapy (Ghofrani, Seeger, & Grimminger, 2005). Imatinib has reduced the severity of symptoms in this patient, including improved haemodynamic parameters and the walking distance. The follow-up proof of concept study has suggested that PAH patients with the highest pulmonary vascular resistance would benefit from the TK inhibitor (TKI) therapy (Ghofrani et al., 2010). Unfortunately, the TKI therapy was associated with the appearance of SAEs (serious adverse effects) such as vomiting, peripheral oedema, and subdural haematomas. The phase III IMPRES study, which also investigated the effects of imatinib on PAH, has demonstrated improvement in walking distance, reduction of pulmonary resistance, and improvement in cardiac output (Hoepner et al., 2013). Although impressive results have been obtained in experimental models of PH (Schermuly et al., 2005) and in the compassionate treatment (Frost et al., 2015; Ghofrani et al., 2005; Speich et al., 2015), the clinical trials were discontinued due to those safety issues of imatinib (Hoepner et al., 2013; Prada, Gavilanes, & Souza, 2015). Furthermore, imatinib inhibits Abl kinase resulting in increased endoplasmic reticulum stress, leading to mitochondrial dysfunction (Rainbolt, Saunders, & Wiseman, 2014).

Nevertheless, it was clearly demonstrated that this type of therapy, that is, targeting the hyper-proliferative phenotype of pulmonary vascular cells in P(A)H, might be exploited especially for patients that had previously be categorized to be incurable with the known standard drugs. For these reasons, testing of inhibitors for other receptor kinases was performed. Here, targeting of EGF receptor (EGFR), fibroblast-derived growth factor receptor (FGFR), vascular endothelial cell-derived growth factor receptor (VEGFR), insulin-like growth factor receptor (IGF-1R), and Src kinases or multi-kinase approaches have been investigated.

In light of the postulated cardiac paradigm of severe PAH (Pulimsetti, Savai, Seeger, & Goncharova, 2017), several multi-targeted tyrosine-kinase inhibitors approved as promising cancer therapeutics were tested in the setting of PH and RV pressure overload. Sorafenib, a multi-kinase inhibitor that among others inhibits PDGFRs, VEGFRs, Flt3, c-Kit, c-Raf, and b-Raf as well as sunitinib through inhibition of PDGFRs, VEGFRs, Flt3, KIT, CSF1R, and RET, both improved RV function by inhibiting pro-fibrotic and hypertrophic myocardial remodelling processes when administered over a short 2-week time period in a PA banding rat model with establish RV hypertrophy (Kojonazarov et al., 2013). But an association with clinical cardiac toxicity has been established for both compounds before (Schmidinger et al., 2008). About 3–8% of cancer patients on TK inhibitors with a VEGF component show signs of adverse cardiotoxic events, and a single VEGF-directed monoclonal antibody (bevacizumab) can cause cardiotoxicity (2–4% of patients on bevacizumab; Touyz & Herrmann, 2018), suggesting VEGF signalling as critical component of cardiac homeostasis. Chu and colleagues first reported sunitinib-mediated cardiomyocyte toxicity through mitochondrial swelling in a retrospective analysis of 75 patients with gastrointestinal stromal tumour (Chu et al., 2007), yet the exact molecular mechanisms underlying cardiotoxicity still remain unclear. Physicians have learned to manage toxicities such as those of VEGF inhibitors clinically through the development of dosing schedules that improve tolerability without compromising efficacy (two consecutive weeks on sunitinib treatment; 1-week rest; Bracarda et al., 2017) and individualizing dosing adjustments. Preclinical studies need to adjust those dosing schedules when advancing from proof-of-concept to translational relevant studies. Of note, it has been shown that congestive heart failure which develops under TK inhibitor treatment is generally completely reversible but requires treatment interruption—which can be resumed after recovery (Schmidinger, Larkin, & Ravaud, 2012). After positive animal studies (Kojonazarov et al., 2013), a phase Ib trial was initiated with the aim to evaluate the safety of sorafenib...
for treatment of PAH (Gomberg-Maitland et al., 2010). Within 16 weeks of observation on 22 study participants, sorafenib demonstrated a safe profile without the appearance of serious adverse effects but no significant improvement in walking distance and haemodynamics were observed. However, an important factor in interpretation of these data is that severely ill patients with functional class IV were excluded. Median value of mPAP was 51 mm Hg (range 36–64). So PAH patients most likely to benefit from TKI therapy were not involved in the study. Thus, despite TKIs demonstrated efficacy in animal models of PAH, severe adverse effects limited their use for PAH treatment.

Based on promising preclinical results, a phase II investigating nilotinib (Bcr-Abl, c-Kit, PDGFR-α/β inhibitor) for the treatment of PAH was initiated (ClinicalTrials.gov Identifier: NCT01179737). This trial was terminated because the drug was not well tolerated and higher rates of SAE events (such as hepato-biliary disorders, three cases of PAH worsening out of 12 treated patients) were observed in the treatment group. Indeed, nilotinib prolonged QT interval (Lu et al., 2012).

In the context of PH and RV pressure overload, de Raaf and colleagues tested the effects of BIBF1000, a nintedanib analogue that targets VEGFRs, PDGFRs, and FGFRs, in a PA banding rat model and demonstrated that 35 days of treatment with a clinically relevant dosage had no detrimental effects on either RV morphology or function (de Raaf et al., 2016). Unfortunately, limited therapeutic success was observed in clinical settings for nintedanib (Richter et al., 2018) as well as for dasatinib (Bcr-Abl, c-Kit, PDGFR-α/β, and Src inhibitor) (Guignabert et al., 2016; Weatherald, Chumais, Savale, et al., 2017) due to endothelial dysfunction.

Indeed, cancer patients show a clear prevalence for cardiovascular events when treated with kinase inhibitors (reviewed in Totzeck, Mincu, Mrotzek, Schadendorf, & Rassaf, 2018; Weatherald, Chaumais, & Montani, 2017). Dasatinib caused cardiomyocyte damage due to off-target binding (Hasinoff, Patel, & Wu, 2017). Clinical data from cancer patients further point towards a better safety profile for nintedanib when compared to sunitinib (Eisen et al., 2015), likely because of different affinities of the inhibitors to the targeted TKs such as VEGFRs. This is important as target affinity and “on- and off-target” toxicities further define the cellular function of the respective kinases and need to be taken into consideration when re-purposing cancer drugs. But lapatinib appears as to be the safest substance among TKIs in terms of cardiotoxicity as demonstrated by recent meta-analysis of clinical trials (Rahmani, Shahrniari, Sheikhi, Ebadi, & Davoodzadeh, 2017).

Along the same line, a key role for signalling through the receptor TK IGF-1R in tumour biology has been postulated and specific inhibitors were generated (King, Alekis, Haluska, & Macaulay, 2014). Shi et al. tested the effects of GSK1904529A, a specific IGF-1R inhibitor, on RV structure and function in a PA banding mouse model (Shi et al., 2016). Therapeutic administration after RV dysfunction and hypertrophy was established, improved RV function and decreased RV hypertrophy, pointing towards a role for IGF-1R signalling in hypertrophic remodelling of cardiomyocytes.

### 4 MAPK SIGNALLING

Intracellular kinases which are placed downstream of growth factor receptor kinases came into the focus of research on new therapeutic strategies. Some of them are directly involved in mediating the growth stimuli from the previously mentioned growth factor receptor kinases to the level of transcriptional control. p38 MAPK inhibitors were initially developed for the treatment of inflammatory and rheumatoid diseases. Targeting p38-MAPK became an interesting therapeutic option for PH since this would target both remodelling and inflammatory pathways in the pulmonary vasculature. Pharmacological inhibition of the p38-MAPK in adventitial fibroblasts showed impressive effects in vitro and in vivo models of pulmonary hypertension (Church et al., 2015). Here, the compound PH-797804 was able to prevent and reverse the pulmonary hypertensive phenotype. The authors further identified IL6 as a main pathogenic cytokine negatively affected by PH-797804 which taken together led them to the conclusion that inhibition of p38-MAPKα targets both remodelling and inflammatory pathways in pulmonary vascular disease. Furthermore, PH-797804 led to improved lung function and reduced dyspnoea in COPD patients (MacNee, Allan, Jones, De Salvo, & Tan, 2013). In the study, the patients were randomized to placebo, 0.5, 3, 6, or 10 mg or PH-797804 treatment. Interestingly, the dosage of 6 mg daily was associated with clinical improvement at 6 weeks of treatment, but not the dosage of 10 mg. PH-797804 has demonstrated a good safety profile with the most commonly reported adverse effect being acneform rash. No liver problems were reported (MacNee et al., 2013). The inflammation markers were not measured in the study. Losmapimod, a p38-MAPK inhibitor developed by GlaxoSmithKline, tested for treatment of COPD patients, did not reduce markers of inflammation measured as sputum neutrophil count, circulating levels of IL-6, IL-8, and C-reactive protein (Lomas et al., 2012). p38-MAPK signalling is involved in the pathobiology of atherogenesis, plaque destabilization, and maladaptive remodelling after myocardial infarction. Therefore, losmapimod has been tested for treatment of patients with acute myocardial infarction (O’Donoghue et al., 2016). Losmapimod has reduced the concentration of C-reactive protein and the incidence of liver enzyme abnormalities and of infections were similar by the treatment groups. However, no reduction in the risk of recurrent major adverse cardiovascular events was observed. Therefore, the development of losmapimod for the myocardial infarction treatment was stopped. SCIO-469 (talmapimod), another p38-MAPK inhibitor tested for the treatment of the rheumatoid arthritis, has demonstrated only transient improvement in the markers of the disease activity, additionally it caused unacceptable side effects including liver toxicity and skin reactions (Genovese et al., 2011). Therefore, the clinical development of the substance was discontinued.

Along the same line, an MAPK upstream of the p38-MAPK cascade, Ask1 (apoptosis signal-regulating kinase 1), was targeted with a small molecule inhibitor (GS-444217) to halt disease progression in experimental PH models (Budas et al., 2018). Ask1 belongs to the class of ubiquitously expressed MAP 3K (MAPK kinase kinase) and is
activated in response to oxidative stress. Upon activation, Ask1 phosphor-
ylates MAPKs which in turn phosphorylate and activate effec-
tor MAPKs, such as p38-MAPK and JNK. In a recent study, GS-
444217 blocked p38-MAPK phosphorylation and reduced pulmonary
vascular remodelling, decreased pulmonary arterial pressure, and
reduced right ventricular hypertrophy in PH models with established
disease (Budas et al., 2018). In a PA banding mouse model, selective
inhibition of Ask1 with GS-444217 (a selonsertib analogue) as well as
p38-MAPK via PH-797804 improved RV function through a robust
reduction in RV fibrosis when administered once RV dysfunction and
hypertrophy was established (Budas et al., 2018; Kojonazarov et al.,
2017). Altogether, these data point towards a pro-fibrotic role for
MAPK signalling through p38-MAPK and highlight the anti-fibrotic
potential of MAPK inhibitors in the pressure overloaded RV. The role
of MAPK signalling in the hypertrophied RV has recently attracted
attention in PAH as a phase II clinical trial with selonsertib was con-
ducted (Rosenkranz et al., 2017). Here, selonsertib at the dosages of
2, 6, and 18 mg was tested for PAH treatment (ClinicalTrials.gov Iden-
tifier: NCT02234141). Selonsertib did not change the pulmonary vas-
cular resistance (primary endpoint) and 6-min walk distance
(Rosenkranz et al., https://erj.ersjournals.com/content/50/suppl_61/
OA1983; Boucherat, Provencher, & Bonnet, 2018). No significant
change was observed in the markers of cardiac function: NT-proBNP,
cardiac index, the right ventricular cardiac power, or the right ventric-
ular performance index. The numbers of reported SAE was higher in
the groups of patients treated with selonsertib 6 mg and 18 mg, being
17 cases in 49 patients (17/49; 34.69%) in the 6-mg treatment group
and 16/47 (34.04%) in 18-mg treatment versus 7/37 (18.92%) in the
 placebo group. SAE included haemorrhagic anaemia (two cases—one
case in 6-mg group and one case in 18-mg group), RV failure (four
cases in 6-mg and one case in 18-mg vs. one case in placebo
group), and pneumonia (three cases in 6-mg and two cases in 18-mg
groups vs. no cases in placebo group). In a phase II trial testing
selonsertib for treatment of nonalcoholic steatohepatitis, no heart-
related SAE were reported (Loomba et al., 2017). This might suggest
that compromised RV in PAH could be prone to effects of the inhibi-
tor. Indeed, increased Ask1 activity has been demonstrated in the
stressed RV (Budas et al., 2018) and in LV (Nako et al., 2012).

5 | JAK–STAT SIGNALLING

JAK/STAT is a signalling pathway regulating immunity, cell division,
and cell death, whose importance has been recognized in PAH patho-
biology (Paulin, Meloche, & Bonnet, 2012). It recently became an
attractive drug target as it was shown to be deregulated in a broad
range of fibrotic disorders such as myelofibrosis. Therefore,
ruxolitinib, a Jak1/2 inhibitor, is approved by the FDA for the treat-
ment of myeloproliferative neoplasms like essential thrombocytopenia,
polycythaemia vera, and myelofibrosis. Jak2 is a major regulator of
cytokine and growth factor stimulation leading to the downstream
activation of Stat3 signalling and also to the activation of the non-
canonical PI3K as well as the MAPK cascade, all of which are involved
in the pathogenesis of idiopathic pulmonary fibrosis (IPF) and PH
(Paulin et al., 2012). This makes Jak2 an ideal kinase for specific inhibi-
tion by compounds like ruxolitinib or JSI-124 (Milera et al., 2018). The
authors showed that Jak2 is activated in pulmonary arteries from idio-
pathic fibrosis (IPF) patients, which is further increased in PH associ-
ated to IPF (PH-IPF) and that Jak2 inhibition ameliorates pulmonary
artery remodelling, increases pulmonary artery relaxation, and
improves the overall symptoms of the disease in this experimental
model of bleomycin-induced IPF with PH. Interestingly, ruxolitinib has
been reported to improve pulmonary hypertension in patients with
myelofibrosis (Tabarroki et al., 2014). It reduced right ventricular
pressure (measured by echocardiography), elevated NO, and decreased
NT-proBNP. Of note, a case of PAH worsening in a myelofibrosis
patient treated with ruxolitinib has been described (Low, Howard,
Harrison, & Tulloh, 2015). In a peptide-based kinase-activity screen,
Jak2 was identified as highly active in PAH-patient derived PASMCs
compared to healthy cells. Jak2 inhibition by ruxolitinib led to an
improvement of cardio-pulmonary function in two independent ani-
mal models of P(A)H via blockage of the Jak2-Stat3 signalling pathway
(Dinesh Yerabolu, Astrid Weiss, Ralph Schermuly, May 15th 2019,
personal communication). Jak/STAT is an important signalling path-
way involved in homeostasis in many organ systems indicating a high
risk for unwanted side effects. Many adverse effects upon Jak/STAT
inhibition have been described, such as anaemia, agranulocytosis,
thrombocytopenia, gastrointestinal disturbances, and neurotoxicity
(Tefferi & Pardanani, 2011). The high rate of the treatment associated
adverse effects limits the use of ruxolitinib for myelofibrosis therapy
(Verstovsek, 2009). CEP-701 (lestaurtinib), XL019, and TG101348 are
other Jak inhibitors in an early clinical phase of development
(Verstovsek, 2009).

6 | CYCLIN-DEPENDENT KINASES

Very recently, cyclin-dependent kinases (CDKs) were identified as
potential drug target in patients with IPAH (Weiss et al., 2019).
CDKs together with their corresponding cyclin orchestrate the
complex events that drive the cell cycle. Transition to the next
phase depends on the activity of CDK1, CDK2, CDK4, and CDK6
all of which phosphorylate and inactivate the Rb (retinoblastoma
protein), a tumour suppressor protein that limits the transcription
of E2F proliferative target genes. The activity of the CDK-cyclin
complexes is often deregulated in cancer cells leading to uncon-
trolled cell growth due to increased Rb phosphorylation (i.e., its
inactivation) and subsequent enhanced transcriptional E2F activity.
Palbociclib, targeting CDK4 and CDK6, has been recently approved
for the treatment of advanced breast cancer. The authors could
show that CDK4 and CDK6 inhibition by palbociclib reduced prolif-
eration via cell cycle arrest and interference with the downstream
CDK-Rb-E2F signalling pathway in pulmonary vascular cells (Weiss
et al., 2019). In vivo, palbociclib was able to reverse the pathologi-
cal disease phenotype by a reduction in right ventricular systolic
pressure, reduction in right heart hypertrophy, restoration of the
cardiac index, and a reduction pulmonary vascular remodelling. These results clearly illustrate that CDK inhibition by palbociclib can provide an additional therapeutic option in the treatment of PAH (Weiss et al., 2019). Besides palbociclib, several other CDK inhibitors are currently in the development such as dinaciclib, milciclib, riviciclib, romociclib, seliciclib, and voruciclib, which differ in their activity and target profile (Galbraith, Bender, & Espinosa, 2019). Their most common adverse effect is myelosuppression causing development of anaemia, infections, and thrombocytopenia (Loibl et al., 2017). Reversible neutropenia was reported as a most common adverse effect of dinaciclib (Mita et al., 2017). For the milciclib, neutropenia and thrombocytopenia were reported as the most common adverse effect (Aspeslagh et al., 2017). No cardiotoxic effects were reported for palbociclib. When administered at the recommended dosing, it did not cause prolonged QT intervals to a clinically relevant level (Durairaj et al., 2018). Roniciclib was tested for the treatment of different kinds of solid tumours (Bahleda et al., 2017; Lin, Lin, Hsueh, Chou, & Wong, 2018; Reck et al., 2019). All of the listed drugs are novel substances; thus, more information is awaited from the currently running studies about their efficiency and toxicity.

7 | TGF RECEPTOR SIGNALLING

In mammals, seven type I (also known as activin receptor-like kinases [ALKs]) and five type II receptors belong to the superfamily of TGF-β receptors (intensively reviewed in Rol, Kurakula, Happe, Bogaard, & Goumans, 2018). Of all receptors belonging to the TGF-β superfamily, the bone morphogenetic protein type 2 receptor (BMPR2) is potentially the most relevant one for PAH. More than 70% of patients with familial PAH and 20% of patients with idiopathic PAH have heterozygous mutations that interfere with BMPR2 kinase function. These mutations can lead to a loss-of-function phenotype associated with a higher disease severity (Evans et al., 2016). Id proteins are transcriptional targets of active BMPR signalling and function as dominant-negative transcriptional regulators. In the absence of ligands (e.g., BMP and GDF), the pathway is maintained in an inactive status by FKBP12 which shields serine and threonine residues of BMPR1 from being phosphorylated by BMPR2. Once activated, the transcriptional targets, Id1, Id2, and Id3, are all expressed by PAECs and PASMcs underlining their critical role in the tight regulation of cellular differentiation and proliferation. In the case of mutated BMPR2, there is a reduction in Id gene transcription which results in a loss of the growth suppressive effects of BMPs. Hence, the therapeutic approaches aim to reconstitute the kinase function of BMPR2 to regain cell cycle control. Recently, tacrolimus (FK506) was successfully tested in a phase Ila trial in PAH patients which showed that the compound is well tolerated and that BMPR2 levels could be increased in a subset of patients (Spiekerooeter et al., 2017). This further supports the authors’ previous encouraging results obtained in two animal models of PH in which a reduction in right ventricular systolic pressure and right ventricular hypertrophy was observed (Spiekerooeter et al., 2013). In terms of the mode of action, it is still not clear how precisely tacrolimus restores/activates BMPR2 levels. Similarly to BMP-induced pathways, TGF-β signalling is very complex and multifactorial. Here, although TGF-βRII/TGF-βRI is the preferable high affinity signalling complex, in endothelial cells, TGF-β can also signal through TGF-βRII/ALK1/ALK5. In idiopathic PAH, levels of ALK1 and endoglin (membrane glycoprotein) are specifically increased in PAECs, leading to enhanced Smad1/5 phosphorylation when stimulated with TGF-β (Gore et al., 2014). In the context of PAH, until now, only a limited number of kinase inhibitors interfering with TGF-β receptor activation, specifically the ALK5 kinase (TGF-βRII), were applied in animal models of PH. SD-208 (Zaiman et al., 2008) as well as SB525334 (Thomas et al., 2009) significantly attenuated the development of the PH and inhibited right ventricular hypertrophy in a rat model of PAH. SB525334 inhibited the ex vivo TGF-β induced proliferation of the PASMC isolated from PAH patients and reduced vascular remodelling in MCT model (Thomas et al., 2009). Currently, many substances (including monoclonal antibodies) targeting TGF-β or its receptors are at the preclinical or early clinical stage of development (Haque & Morris, 2017). LY2157299, a small molecule TGF-β inhibitor was tested in a first-in-human dose study and demonstrated a good safety profile with no cardiovascular toxicities and no tumourigenesis (Rodon et al., 2015).

8 | G-PROTEIN COUPLED RECEPTOR SIGNALLING

Smooth muscle as well as endothelial cells in the pulmonary vasculature express GPCRs which mediate signalling events induced by vasoactive mediators such as endothelin, angiotensin II, serotonin, prostacyclin, and vasoactive intestinal peptide (as summarized in Lynikkel & Murray, 2018). GPCRs reside in the plasma membrane and serve as intracellular docking station for heterotrimeric G proteins (composed of Ga and Gβγ subunits; Alexander, Christopoulos, et al., 2017). Extracellular ligand binding induces a conformational change resulting in the (in)activation of downstream signalling pathway dependent on the particular type of the Ga subunit. Once activated by an exchange of GTP for GDP on the Ga subunit, the heterotrimeric complex dissociates with Ga being the main effector molecule of this signalling mechanism (Viswanathan, Mamazhayakovp, Schermuly, & Rajagopal, 2018). The RhoA and Rho-kinase (ROCK) are downstream target proteins of the GPCR-mediated activation of Ga12/13 cascade in response to extracellular levels of serotonin, thrombin, and sphingosine. ROCK activation is responsible for the phosphorylation of the myosin light chain leading to actin-myosin cross-bridging and subsequent cellular contraction. Therefore, the non-selective ROCK inhibitor, fasudil, serves as a potent inhibitor interfering with the vaso-constrictive phenotype of PASMCs in PAH. Fasudil was effective in reducing pulmonary artery pressure and improved survival in experimental models of
PH, and in clinical trials, both pulmonary artery pressure and pulmonary vascular resistance were significantly decreased by fasudil without apparent side effects (Abe et al., 2004; Abe et al., 2006; Jiang et al., 2014; Kojonazarov, Myzzaakhmatova, Sooronbaev, Ishizaki, & Aldashev, 2012; Zhang & Wu, 2017). Fasudil is approved in Japan for the treatment of cerebral vasospasm induced by subarachnoid haemorrhage. Its acute vasodilator effects have been shown in the patients with severe IPAH (Fukumoto et al., 2005). In the study, intravenous administration of fasudil caused an acute reduction in PVR and an increase in cardiac output. These observations were confirmed in another study (Ishikura et al., 2006) but only a non-significant decrease in systemic pressure was observed. Selective pulmonary vasodilative effect of fasudil was observed in the Kyrgyz highlanders suffering from hypoxia-induced PAH (Kojonazarov et al., 2012). When compared with the aerosolized iloprost, fasudil was as efficient as iloprost in PVR reduction, but caused more pronounced increase in cardiac output (Jiang et al., 2014). Whether the observed difference is attributed to the direct cardiac effects of fasudil remains open. In fact, recent findings suggest that disturbances in ROCK signalling contribute to heart failure and that ROCK inhibition restores calcium handling in myocardium normalizing cardiac contractility (Olgar et al., 2017). In a model of PAH, Rho kinase has been shown to mediate right ventricular systolic dysfunction (Gosal et al., 2015). The observed beneficial haemodynamic effects of fasudil suggest it to be, potentially, an interesting agent to treat right heart failure in the settings of exaggerated PAH. Indeed, intravenous fasudil treatment improved in-hospital mortality and the 30-day re-hospitalization rate of patients with right heart failure due to severe pulmonary hypertension (Jiang et al., 2015). In the double-blinded placebo-controlled study, 10 PAH patients were treated with fasudil for 3 months, and it did not significantly reduce PVR but improved cardiac index (Fukumoto et al., 2013). In all the cited studies, fasudil was well tolerated. However, its long-term effects on the right heart and the metabolic costs of its possible, cardiac stimulating effects are unknown. A study suggests that it might have beneficial effects on cardiac perfusion by relieving coronary microvascular spasm (Mohri, Shimokawa, Hirakawa, Masumoto, & Takeshita, 2003). Azaindole-1 showed similar effects in hypoxia and MCT-induced PH but also reduced right ventricular hypertrophy (Dahal, Kosanovic, et al., 2010). Very recently, a new inhibitor, named compound 3, has been shown to be more potent and highly selective for ROCK-1 and 2 to improve haemodynamic parameters and counteract pulmonary vascular remodelling in experimental PAH (Cantoni et al., 2019). Thus, fasudil and other ROCK inhibitors deserve further investigation with regards to their effects on the right ventricle in PAH.

The sphingosine kinase 1 (SphK1) is another example for an interesting, potentially druggable kinase of the GPCR-induced signalling network. The intracellular SphK1 catalyses the formation of S1P (sphingosine-1-phosphate), a lipid messenger with intracellular and extracellular functions. S1P can be released from pulmonary vascular cells and might act in an autocrine manner via the S1P receptor (GPCR). The SphK1 signalling pathway can regulate expression of Stat3 (increased) and BMPR2 (decreased) thereby building a potential overlap with known PAH disease causing cellular mechanisms. An initial study with SLP7111228, a SphK1 inhibitor, demonstrates effectiveness to improve both cardiac and total pulmonary artery resistance index but without affecting RVSP or RVH in Su/Hox rats (Gairhe et al., 2016). To the contrary, another SphK1 inhibitor, named SKI-II, prevented hypoxia-mediated PH in rats including right ventricular hypertrophy (Chen et al., 2014) which overall shows the discrepancy of results obtained by different experimental models. An excellent review article highlights the possibility of Sphk1 inhibitors for the therapy of PAH (Pyne & Pyne, 2017). But the authors point out the open question about the opposing roles of Sphk1 in PASMCs contractility and preservation of PAECs function due to the different receptors for S1P, namely, S1P1 or S1P2, respectively. In addition, they provide a summary of a study with the SphK1 inhibitor, PF-543, which prevents maladaptive hypertrophy in a hypoxic mouse model of pulmonary hypertension and cardiac remodelling after myocardiad infarction via a reduction of p53 levels. But further investigations are needed to clearly state if sphingosine kinase 1 is an appropriate drug target for PH.

9 | MTOR SIGNALLING

Mammalian target of rapamycin (mTOR) is a major regulator of cellular metabolism, proliferation, and survival that is implicated in various proliferative and metabolic diseases (Goncharova, 2013). Its diverse functions are carried out by to the formation of two distinct multi-protein complexes, namely, mTOR complex 1 (mTORC1) and mTORC2. The mechanisms of activation and downstream signalling and diverging points with other signal transduction pathways are not fully characterized in detail. With a focus on mTOR in pulmonary hypertension, it was shown that this pathway is implicated in the structural remodelling of the pulmonary vasculature mostly by supporting cell growth of PASMCs, PAECs, and fibroblasts. Here, mTORC1 phosphorylates its downstream targets p70 S6 kinase 1 (S6K1) and 4E-binding protein 1 (4E-BP1) leading to activation of ribosomal protein S6 and eIF2E, protein synthesis, and cell proliferation. In vivo, rapamycin (sirolimus) attenuates pulmonary vascular remodelling and right ventricular hypertrophy in hypoxia-induced PH in mice mainly due to anti-proliferative effects on pulmonary vascular cells as well as an anti-hypertrophic mode of action on cardiomyocytes (Paddenberg et al., 2007). In a safety and efficacy pilot trial, everolimus (structurally related to rapamycin) led to a significant reduction in pulmonary vascular resistance (PVR) and to an increase in 6-MWD without causing severe side effects (Seyfarth, Hammerschmidt, Halank, Neuhaus, & Wirtz, 2013). Out of 10 patients enrolled, one patient developed signs of right heart failure after 5 months of treatment, and the medication was stopped. Another patient developed acute bronchitis, probably due to immunosuppression by everolimus, but the peripheral blood cell count did not change significantly during the
study. Six patients experienced cholesterol level elevation, and another three patients had elevated triglyceride levels. After 6 months of everolimus treatment, the majority of the patients demonstrated a 15% to 54% improvement in PVR. This change was accompanied by an improvement in cardiac output and in mPAP but the walking distance did not change significantly. Of note, the patients involved into the study were unresponsive to the standard medication. Overall, everolimus was well tolerated by PAH patients, and the adverse effects observed are comparable with those reported on the transplantation patients (Vitko et al., 2005). Despite the common acceptance of mTOR activation in pulmonary vessel remodelling in PAH, only recent data (summarized in Houssaini & Adnot, 2017) highlight its function in the right ventricle especially during cardiac adaptation to pressure overload by compensatory myocardial hypertrophy. Very recently, it was revealed that both mTORC1 and mTORC2 are up-regulated in remodelled pulmonary arteries in an experimental model of PH, while only mTORC1 was activated in the remodelled right ventricle. Targeted mTORC1 and mTORC2 inhibition by PP242 led to an improvement of cardio-pulmonary function including right ventricular hypertrophy without affecting the survival of cardiomyocytes (Pena et al., 2017). There data are encouraging and strongly promote the concept of dual targeting of aberrant mTOR signalling which is responsible for the pathological cellular phenotype in the pulmonary vasculature as well as in the right ventricle in PAH. Another phase I clinical trial investigating the effect of an albumin-bound mTOR inhibitor (ABI-009, nab-sirolimus) in patients with severe PAH was initiated in 2017, and first data are expected in August 2019 (ClinicalTrials.gov Identifier: NCT02587325).

10 | GLUCOSE METABOLISM

Beside kinases involved in the hyper-proliferative or contractile phenotype of pulmonary vascular cells in PH, scientists started to investigate other cellular mechanisms like the dysfunction of the mitochondrial glucose oxidation (Michelakis et al., 2017). Here, cancer-like proliferating PASMCs, PAECs, and fibroblasts in PAH show a metabolic switch from mitochondrial glucose oxidation to glycolysis for the purpose of ATP production. As a consequence of mitochondrial suppression, those cells exhibit a resistance towards mitochondria-dependent apoptosis to a substantial extent. In pulmonary arteries of PAH patients, this resistance is characterized by an increase in pyruvate dehydrogenase kinase (PDK), which in turn is a negative regulator of the mitochondrial enzyme pyruvate dehydrogenase (PDH), the gatekeeping enzyme of glucose oxidation. Michelakis et al. have intensively studied the role of PDH in PAH (Michelakis et al., 2002). In their recent work, they investigated the therapeutic use of dichloroacetate (DCA), a PDH kinase inhibitor, in PAH patients (Michelakis et al., 2017). In this dose-finding study, the patients were treated with 3.0 mg/kg BID, 6.25 mg/kg BID, or 12.5 mg/kg BID DCA. DCA caused an improvement in haemodynamic parameters and functional capacity. However, the patients demonstrated a heterogeneous response to the therapy. A correlation between the clinical response to DCA and a single nucleotide polymorphism (SNP) score was found in a post hoc analysis. The SNP score included known inhibitory SNPs of sirtuin 3 (Sirt3) and of uncoupling protein 2 (Ucp2), which are upstream regulators of PDH. The authors hypothesized that the presence of these inhibitory SNPs inactivates PDH and makes it non-responsive to DCA. Overall, DCA was well tolerated; but peripheral neuropathy was observed at the highest dose of 12.5 mg/kg BID DCA, which disappeared within 1–3 months after treatment discontinuation. This type of reversible neuropathy is a known adverse effect of DCA (Berendzen, Theriaque, Shuster, & Stacpoole, 2006). This study supports the rationale of pursuing PDK inhibition in PH patients with functional Sirt3 and Ucp2 variants, further supporting the concept of individualized medicine for PAH on the basis of restoration of a normal mitochondrial phenotype including energy metabolism, controlled proliferation, and apoptosis sensitivity.

Piao and colleagues also demonstrated a beneficial effect of pyruvate dehydrogenase kinase (PDK) inhibition with dichloracetate on right ventricular function in pulmonary artery banded rats (Piao et al., 2010). DCA was shown to increase the pyruvate flux into mitochondria and shift the metabolism from glycolysis to glucose oxidation to prevent a metabolic switch where mitochondrial glucose oxidation is suppressed (Michelakis, Webster, & Mackey, 2008). Preventive DCA treatment improved cardiac output, reduced the degree of RV hypertrophy, and decreased RV systolic pressures through better oxygen consumption, likely in cardiomyocytes, pointing towards improved contractile RV function (Piao et al., 2010). Metabolic reprogramming was further linked to signalling through the PI3K–Akt–mTOR axis by regulating glycolytic metabolism and thereby driving cancer progression (Lien, Lyssiotis, & Cantley, 2016). mTOR is the hub of this pathway and downstream signalling can be effectively blocked with rapamycin. Rapamycin additionally has prominent anti-inflammatory properties, and PA banded rats treated with rapamycin developed less hypertrophy but similar RV systolic pressures compared with respective controls, further suggesting an improvement in contractile RV function (Harston et al., 2011).

11 | OTHER DRUGS FOR THE TREATMENT OF PAH

In contrast to the previously mentioned kinase inhibitors, at least one compound with an unknown set of target structures has been described to exert a therapeutic effect in the treatment of P(A)H. In vitro, pirfenidone showed anti-proliferative, anti-migratory, and anti-fibrotic properties in PASMCs derived from IPAH patients (personal communication). Mechanistically, pirfenidone promoted nuclear translocation of the transcription factor Foxo1 (forkhead box O1) in human IPAH-PASMCs and treated Su/Hox rats. Although the details of the mode of action for pirfenidone are yet still unclear, this drug showed impressive results in terms of partial reversal of established pulmonary hypertension with a reduction in
In contrast to previously discussed kinase inhibitors, pirfenidone is in clinical use for longer time, and more is known about its efficiency and safety. Currently, it is approved for the treatment of idiopathic pulmonary fibrosis (IPF). In phase II clinical trials assessing pirfenidone in patients with IPF, it reduced lung function decline, slowed disease progression, and reduced mortality (Nathan et al., 2017). Beside multicentre randomized clinical trials, pirfenidone’s efficiency was confirmed in so-called “real-world” studies from centres treating IPF patient (Oltmanns et al., 2014). The most commonly reported adverse effects of pirfenidone are rash and dysfuction of the gastrointestinal system (Lancaster et al., 2017). Pirfenidone is being tested also for treatment of rheumatic disorders with promising results (Walker & Margolin, 2001). No reports on cardiotoxicity induced by pirfenidone were found in the available literature (Bai, Yang, Liu, Ning, & Zhang, 2019). Considering an important contribution of myocardial fibrosis to cardiac failure, a study was initiated to investigate pirfenidone for the treatment of patients with heart failure with preserved ejection fraction (HFpEF; ClinicalTrials.gov Identifier: NCT02932566). The study is based on the experimental studies demonstrating anti-fibrotic effect of pirfenidone in the heart of IPF patients (Crnkovic et al., 2018; Wang, Wu, Chen, Zhao, & Li, 2013). In line with this, pirfenidone (targeting p38-MAPK, TGF-β1 and others are discussed Moran, 2011), modestly reduced RV fibrosis without affecting RV function in PA banded mice (Crnkovic et al., 2018). If a positive effect of pirfenidone on cardiac structure and function is observed in the clinical trial, this would make pirfenidone an extremely interesting candidate for the treatment of PAH patients since it would efficiently target both vascular and cardiac remodelling in PAH (Poble et al., 2019).

12 | SUMMARY

The human kinome contains 518 members constituting about 1.7% of all human genes, and to date, 48 small molecule protein kinase inhibitors have been approved by the FDA for the treatment of various diseases (Roskoski, 2019). Twenty-five kinase inhibitors target receptor protein-TKs, 10 are non-receptor protein-TK inhibitors, and 13 inhibit protein-serine/threonine protein kinases. Most of the small molecule inhibitors predominantly interact directly with the protein kinase domain of the following kinases: ALK, B-Raf, BCR-Abl, EGFR, and VEGFR. As there is an intense overlap between signalling pathways crucial for the development for cancer malignancies and the abnormal phenotype of pulmonary vascular cells, several kinase inhibitors have been investigated for their therapeutic potential in pulmonary hypertension, namely, imatinib, erlotinib, gefitinib, lapatinib, dacomitinib, nintedanib, sunitinib, sorafenib, dasatinib, nilotinib, ruxolitinib, palbociclib, everolimus, and sirolimus. Some of those inhibitors have already been tested in clinical trials or compassionate treatment settings for PH (Table 2), and still, there are many more which hold promise for future applications. Out of the list provided in Table 1, the following compounds showed promising results in experimental models of PH and/or in other human diseases. They are of great interest for new PH treatment options. PH-797804 is a novel pyridinone inhibitor of p38-MAPKα and p38-MAPKβ (fourfold less selective). p38-MAPK is one of the central downstream signalling hubs which is triggered by many growth factors and cytokines. Its activity is often increased in several pulmonary vascular cell types, and its targeted inhibition showed impressive improvements in cardio-pulmonary function in various animal models without obvious signs of cytotoxicity. The IL6-Jak–Stat is an intracellular pathway which recently gained a lot of attention in the community of PH researches: A humanized monoclonal antibody, tocilizumab, improved PAH symptoms in a patient with mixed connective tissue disease and severe PAH (Furuya, Satoh, & Kuwana, 2010), and an open-label study has been launched to assess its efficacy and safety in PAH patients (ClinicalTrials.gov NCT02676947). Our own in vivo data demonstrated beneficial effects upon blockade of Jak (a crucial downstream kinase of the activated IL-6/IL6R/gp130 complex) by ruxolitinib, a drug which is approved for myeloproliferative neoplasm (personal communication). In the same line of confidence, palbociclib (targeting CDK4 and CDK6), an FDA-approved compound for the treatment of estrogen receptor- and HER2-positive breast cancer, was successfully evaluated as promising therapeutic strategy for PH (Weiss et al., 2019). LY2157299 (galunisertib) is a TGF-β1 receptor inhibitor, which is under clinical investigations for anti-cancer treatment with a good safety profile without signs of cardiovascular toxicities. Current research also focusses on sphingosine kinases 1 and 2 (SphK) which might become interesting drug targets but need further validation including a precise analysis of the inhibitors’ (SKI-II, PF-543) mode of action concerning their effects on pulmonary vasculature and on the heart (Pyne & Pyne, 2017). Finally, initial data from the clinical phase I study with an albumin-bound mTOR inhibitor (nab-sirolimus, ABI-009) in severe PAH are expected to be published soon (for details see Table 2). Of course, successful application can be limited by ineffectiveness, drug-related but unwanted side effects and/or cardiotoxicity as previously discussed. This demonstrates the necessity for further investigations of existing kinase inhibitors as well as the search for new “druggable” protein kinases. Hopefully, these studies will make a substantial contribution to upcoming therapeutic options in the treatment of PH in addition to other classes of inhibitors (Sitbon et al., 2019).

13 | FUTURE DIRECTIONS

Targeted kinase inhibition is a reasonable therapeutic approach as demonstrated by the efficacy of distinct compounds in experimental models of PH (in Table 1) or initial clinical studies (Table 2). However, TK inhibitor induced severe adverse events that limit their use for the treatment of PAH patients. One approach to improve tolerability and to reduce appearance of adverse effects
| Inhibitor          | Target                      | Animal model       | Effects on the lung | Effects on the right ventricle | Source                                      | Side effects in humans                                                                 |
|--------------------|-----------------------------|--------------------|---------------------|--------------------------------|---------------------------------------------|------------------------------------------------------------------------------------------|
| Imatinib           | Bcr-Abl, c-Kit, DDR, PDGFR-α/β | Hypoxic mice       | ↑ Function          | ↑ Function                     | (Schermuly et al., 2018)                   | Subdural hematoma, CHF, LVD, ER stress                                                    |
|                    |                             | MCT rats           | ↓ Remodeling        | ↓ Hypertrophy                  |                                             |                                                                                          |
|                    |                             | Su5416/Hypoxic rats| ↑ Function          | ↑ Function                     | (Ciulcan et al., 2017)                     |                                                                                          |
|                    |                             |                    | ↓ Remodeling        | ↓ Hypertrophy                  |                                             |                                                                                          |
| Erlotinb           | EGFR                        | Hypoxic mice       | → Function           | → Function                     | Dahal et al. (2012)                        | MI, apoptosis induction for gefitinib, reduction in LVEF for lapatinib                    |
| Gefitinib          | EGFR, ErbB2                 |                    | → Remodeling         | → Hypertrophy                  |                                             |                                                                                          |
| Lapatinib          | EGFR                        | MCT rats           | ↑ Function           | ↑ Function                     | (Yu et al., 2010)                          |                                                                                          |
|                    |                             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             |                                                                                          |
| Dacomitinib        | EGFR                        | MCT rats           | ↑ Function           | ↑ Function                     | (Yu et al., 2010)                          |                                                                                          |
|                    |                             | Su5416/Hypoxic rats| → Function           | → Hypertrophy                  |                                             |                                                                                          |
|                    |                             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             |                                                                                          |
| Nintedanib         | FGFR, PDGFR-β, VEGFR        | Su5416/Hypoxic rats| → Function           | ↑ Function                     | (Rol et al., 2018) (Richter et al., 2018)  |                                                                                          |
|                    |                             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             | Fibrosis                                                                                  |
|                    |                             |                    |                       |                                |                                             |                                                                                          |
| BIBF1000 (Nintedanib analogue) |                    | PAB rats (day 7)    | → Function           | → Fulton index                 | (de Raaf et al., 2006)                     |                                                                                          |
|                    |                             |                    |                       | ↓ CM area                      |                                             |                                                                                          |
|                    |                             |                    |                       | → Fibrosis                     |                                             |                                                                                          |
|                    |                             |                    |                       | → cap. density                 |                                             |                                                                                          |
| Sunitinib          | PDGFR-β, VEGFR, c-KIT, FLT3  | MCT rats           | ↑ Function           | ↑ Function                     | (Kojonazarov et al., 2018)                 | QT prolonged CHF, mitochondrial dysfunction, PAH induction                                |
|                    |                             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             |                                                                                          |
|                    |                             | PAB mice (day 7)    |                       |                                |                                             |                                                                                          |
|                    |                             |                    |                       |                                |                                             |                                                                                          |
| Sorafenib          | b-RAF, c-KIT, FLT3, PDGFR-β  | MCT rats           | ↑ Function           | ↑ Function                     | (Kojonazarov et al., 2018)                 | MI, hypertension, mitochondrial dysfunction, PAH induction                                |
|                    | RAF-1, VEGFR-2/3             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             |                                                                                          |
|                    |                             | PAB mice (day 7)    |                       |                                |                                             |                                                                                          |
|                    |                             |                    |                       |                                |                                             |                                                                                          |
| Dasatinib          | Bcr-Abl, c-Kit, PDGFR-α/β, Src| MCT rats           | ↑ Function           | ↑ Function                     | (Pulamsetti et al., 2010)                  | QT prolonged, CHF, LVD, MI, ER stress, PAH induction                                     |
|                    |                             |                    | ↓ Remodeling         | ↓ Hypertrophy                  |                                             |                                                                                          |

(Continues)
| Inhibitor       | Target                          | Animal model          | Effects on the lung | Effects on the right ventricle | Source                          | Side effects in humans                  |
|-----------------|---------------------------------|-----------------------|---------------------|-------------------------------|--------------------------------|-----------------------------------------|
| Nilotinib       | Bcr-Abl, c-Kit, PDGFR-α/β       | MCT rats              | ↑ Function          | Hypertrophy                   | (Pullamsetti et al., 2010)    | QT prolonged, peripheral artery disease, ER stress |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| GSK1904529A     | IGF-1R                          | Hypoxic mice          | ↑ Function          | Hypertrophy                   | (Shi et al., 2015)            |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | PAB mice (day 7)      | ↑ Function          | Hypertrophy, Fibrosis, nd. cap. density | (Church et al., 2017)      |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | SB203580, PH-797804   | ↑ Function          | Hypertrophy                   | (Kojonazarov et al., 2017)    |                                         |
|                 | p38-MAPKα                       |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | MCT rats              | ↑ Function          | Fibrosis, nd. cap. density    | (Budas et al., 2017)         |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | PAB mice (day 7)      | ↑ Function          | Hypertrophy                   |                                |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | GS-444217, Ask1       | ↑ Function          | Hypertrophy                   |                                |                                         |
| (selonsertib    |                                 |                       | ↓ Remodeling        | Fibrosis, nd. cap. density    |                                |                                         |
| analogue)       |                                 | MCT rats              | ↑ Function          |                               |                                |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | PAB mice (day 7)      | ↑ Function          | Hypertrophy                   |                                |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| JSI-124         | Jak-2                           | Bleomycin-induced fibrosis | ↑ Function          | Hypertrophy                   | (Milara et al., 2018)       |                                         |
|                 |                                 |                       | ↓ Remodeling        | Fibrosis                      |                                |                                         |
| Ruxolitinib     | Jak-1/2                         | Hypoxic mice          | ↑ Function          | Hypertrophy                   | pers. comms.                 |                                         |
|                 |                                 | MCT rats              | ↓ Remodeling        |                               |                                |                                         |
| Palbociclib     | CDK4, CDK6                      | MCT rats              | ↑ Function          | Hypertrophy                   | (Weiss et al., 2005)         |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| SD-208          | TGF-βRI (Alk5)                  | MCT rats              | ↑ Function          | Hypertrophy                   | (Zaiman et al., 2016)        |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| SB525334        | TGF-βRI (Alk5)                  | MCT rats              | ↑ Function          | Hypertrophy                   | (Thomas et al., 2015)        |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| Fasudil         | ROCK                            | MCT rats              | ↑ Function          | Hypertrophy                   | (Abe et al., 2004)           |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
|                 |                                 | Hypoxic mice          | ↑ Function          | Hypertrophy                   | (Abe et al., 2006)           |                                         |
|                 |                                 |                       | ↓ Remodeling        |                               |                                |                                         |
| Azaindole-1     | ROCK                            | Hypoxic mice          | ↑ Function          |                                | Dahal et al. (2004)          |                                         |
|                 |                                 | MCT rats              | ↓ Remodeling        | Hypertrophy                   |                                |                                         |

(Continues)
| Inhibitor                  | Target         | Animal model          | Effects on the lung | Effects on the right ventricle | Source                                                                 | Side effects in humans |
|---------------------------|----------------|-----------------------|---------------------|-------------------------------|-------------------------------------------------------------------------|------------------------|
| Compound 3 ROCK MCT rats  | ROCK           | MCT rats              | ↑ Function          | ↑ Function Hypertrophy        | (Cantoni et al., 2017a)                                                |                        |
| Gallein PAB rats (day 14) | Gβ3-GRK2       | PAB rats              | ↓ Remodeling        | → Function Hypertrophy        | (Piao et al., 2009)                                                    |                        |
| PF-543 SphK1 Hypoxic mice | SphK1          | Hypoxic mice          | → Remodeling        | → Function Hypertrophy        | (MacRitchie et al., 2018)                                              |                        |
| SKII SphK1 Hypoxic rats   | SphK1          | Hypoxic rats          | ↓ Remodeling        | ↑ Function Hypertrophy        | (Chen et al., 2017b)                                                   |                        |
| SLP7111228 SphK1          | SphK1          | Su5416/Hypoxic rats   | ↑ Remodeling        | ↑ Function Hypertrophy        | (Gairhe et al., 2017)                                                  |                        |
| Tacrolimus (FK506)        | FKBP12 BMPR2   | Hypoxic mice          | ↑ Function          | ↑ Function Hypertrophy        | (Spiekerkoetter et al., 2011)                                          |                        |
| Tacrolimus (sirolimus) mTOR | mTOR           | Hypoxic mice          | ↑ Function          | ↑ Function Hypertrophy        | (Paddenberg et al., 2015)                                              |                        |
| Rapamycin (sirolimus)     | mTOR           | Hypoxic mice PAB rats (day 0) | ↑ Function | ↑ Hypertrophy RVSP Fibrosis cap. density | (Harston et al., 2018)                                                 |                        |
| Everolimus mTOR MCT rats  | mTOR           | MCT rats              | ↑ Function          | ↑ Function Hypertrophy        | (Nishimura et al., 2007)                                               |                        |
| PP242 mTOR Su5416/Hypoxic rats | mTOR       | Su5416/Hypoxic rats   | ↑ Function          | ↑ Function Hypertrophy        | (Pena et al., 2013)                                                   |                        |
| DCA PDH kinase (PDK)      | PDH kinase     | MCT rats              | ↑ Function          | ↑ Function Hypertrophy        | (Li, Yan, Shen, Liu, & Ma, 2019)                                       | (McMurtry et al., 2014) |
| Pirfenidone Anti-fibrotic | Anti-fibrotic  | Su5416/Hypoxic rats   | ↑ Function          | ↑ Function Hypertrophy        | (Poble et al., 2010)                                                  |                        |
|                          | Anti-inflammatory |                        | ↑ Remodeling        |                                |                                                                         |                        |
|                          | Anti-oxidative  |                        | ↑ Remodeling        |                                |                                                                         |                        |

(Continues)
would be to decrease the drug dosages or to establish a treatment regimen with intermittent drug administration similar to oncologic studies. In the best case, the same effective dosages of kinase inhibitors as used for oncologic patients should be applied. In the IMPRES study, the patients received 200-mg imatinib once daily with the subsequent up-titration to 400 mg once daily if tolerated (Frost et al., 2015). Whether the observed therapeutic effect correlated with the achieved dosage is unclear due to low patient numbers. A hint supporting the suggestion to treat PAH patients with lower dosages comes from animal studies. In an experimental model of PAH, imatinib was efficient at the dosages of 50 and 100 mg/kg (Schermuly et al., 2005) and 15 and 50 mg/kg (Leong, Okida, Higuchi, Yamano, & Hikasa, 2018). For comparison, in oncological studies, imatinib was used at the dosages ranging from 100 mg/kg (Beppu, Jaboine, Merchant, Mackall, & Thiele, 2004; Irsan et al., 2007) to 200 mg/kg (Basciani et al., 2005; Beppu et al., 2004).

Altogether, various multi-targeting and highly specific kinase inhibitors have been shown to directly affect RV myocardial remodelling and improve functional RV adaptation to pressure overload in preclinical settings. Studies on RV function without interfering afterload alterations are important to reduce the risk of adverse cardiac events when re-purposing kinase inhibitors developed as anti-cancer therapeutics for their use in PAH. Moreover, strengthening the RV intrinsically may prevent and/or delay the development of RV failure in PAH. Because of their ability to block several key signalling pathways in the heart and lung using a single pharmacological active molecule, kinase inhibitors need to be further considered for therapeutic exploitation. Circumvention of adverse events (e.g., cardiotoxicity) can be achieved by inhalation to increase the local drug concentration simultaneous to the reduction of the effective dosage within the systemic circulation (Gessler, 2018). Intermittent administration is another opportunity to limit the risk of side effects as stated previously (Bracarda et al., 2017). Here, the underlying assumption is that aberrant pulmonary vascular cells are affected during the time period of drug administration while the healthy cells of the body do not experience any harm and/or only the latter ones can recover from the compounds burden (so called reversal of cardio-toxicity).

It should be kept in mind that the disease-driving force of an increased kinase activity is the result of a multifactorial process. Epigenetic changes in DNA methylation, histone modifications, and the abundance of non-coding RNAs (like IncRNAs or microRNAs) can account for an over-expression as well as post-transcriptional or post-translational events. Here, a special emphasis lies on protein phosphatases that play an important role on the level of endogenous regulators of kinase activity. They serve as a master switch by removing crucial phosphate residues from their substrate molecules. While protein serine/threonine phosphatases (PPPs) are promiscuous in terms of their substrate selectivity, protein tyrosine phosphatases (PTPs) display a more distinct profile and could be of interest for targeted re-constitution or more easily for inhibition. In the case of PAH, it has been shown that hypoxia leads to
| Inhibitor | Design | Conditions | Identifier | Citation and official title |
|-----------|--------|------------|------------|----------------------------|
| **Imatinib** | Type: interventional (Phase II/III) | - PAH (by RHC)  
- WHO class II–IV | NCT00477269 (CSTI571E2203) | Safety and Efficacy of Imatinib Mesylate in Patients With Pulmonary Arterial Hypertension  
Ghofrani et al. (2010)  
A Randomised, Double-blind, Placebo-controlled Study to Evaluate the Safety and Efficacy of the Six Months Treatment With the Tyrosine Kinase Inhibitor STI571 for the Treatment of Pulmonary Arterial Hypertension (PAH) |
| | Enrolment: 59 participants | | | |
| | Allocation: randomized, intervention | | | |
| | Model: parallel assignment | | | |
| | Masking: double (participant, investigator) | | | |
| | Primary purpose: treatment | | | |
| | Study start date: April 2006 | | | |
| | - PAH (by RHC) with  
- Background therapy with PAH-specific drug in doses to be stable for ≥30 days (except for warfarin and prostanycin analogues)  
- 6MWD ≥ 150 and ≤ 450 m  
- WHO class II–IV | | | |
| | NCT00902174 (CQTI571A2301) | | | |
| | Imatinib (QTI571) in Pulmonary Arterial Hypertension (IMPRES) | | | |
| | Hoefer et al. (2013), Querejeta Roca, Campbell, Claggett, Solomon, & Shah (2015), Querejeta Roca, Campbell, Claggett, Vazir, et al. (2015), Shah et al. (2015) | | | |
| | A 24-week Randomized Placebo-controlled, Double-blind Multi-centre Clinical Trial Evaluating the Efficacy and Safety of Oral QT1571 as an add-on Therapy in the Treatment of Severe Pulmonary Arterial Hypertension: Imatinib in Pulmonary Arterial Hypertension, a Randomized, Efficacy Study (IMPRES) | | | |
| | Enrolment: 202 participants | | | |
| | Allocation: randomized, intervention | | | |
| | Model: parallel assignment | | | |
| | Masking: quadruple (participant, care provider, investigator, outcomes assessor) | | | |
| | Primary purpose: treatment | | | |
| | Study start date: September 2009 | | | |
| | Patients who participated in CQTI571A2301 and completed the week 24 visit of the study protocol, including all Study Completion assessments | | | |
| | Patients who withdrew from the CQTI571A2301 study prematurely for reasons not related to study drug or not related to a safety issue but performed all Study Completion assessments | | | |
| | NCT01117987 (CQTI571A2301E1) | | | |
| | Extension to QT1571A2301 to Evaluate the Long-term Safety, Tolerability and Efficacy of Imatinib in Severe Pulmonary Arterial Hypertension (PAH) (IMPRES Extn) | | | |
| | No corresponding publication | | | |
| | An Extension to QT1571A2301 to Evaluate the Long-term Safety, Tolerability and Efficacy of Oral QT1571 (Imatinib) in the Treatment of Severe Pulmonary Arterial Hypertension: IMPRES Extension | | | |
| | Enrolment: 144 participants | | | |
| | Allocation: non-randomized, intervention | | | |
| | Model: Single group assignment | | | |
| | Masking: none (open label) | | | |
| | Primary purpose: treatment | | | |
| | Study start date: April 2010 | | | |
| | - Patients who completed in CQTI571A2301 and included in the safety population | | | |
| | NCT01392469 (CQTI571A2102) | | | |
| | Pharmacokinetic Effects of QTI571 on Sildenafil and Bosentan in Pulmonary Arterial Hypertension Patients | | | |
| | No corresponding publication | | | |
| | A Non-randomized, Multiple Dose, Three Treatment Period, Open-label, Single Sequence, Single Group Study to Evaluate the Pharmacokinetic Effect of Two Doses of QT1571 (Imatinib) on the Co-administered Drugs Sildenafil and Bosentan in Pulmonary Arterial Hypertension (PAH) Patients | | | |
| | Enrolment: 21 participants | | | |
| | Allocation: Non-Randomized, Intervention | | | |
| | Model: Single Group Assignment | | | |
| | Masking: None (Open Label) | | | |
| | Primary Purpose: Treatment | | | |
| | Study start date: April 2011 | | | |
| | - PAH (by RHC) with PVR > 800 dyne•sec•cm⁻⁵  
- On stable doses of bosentan and sildenafil | | | |
| | NCT01392495 (CQTI571A2102E1) | | | |
| | Frost et al. (2015), Hoeper et al. (2013) | | | |
| | Enrolment: 17 participants | | | |
| | Allocation: included in the safety population | | | |
| | NCT01392469 (CQTI571A2102) | | | |
| Inhibitor      | Type                  | Allocation: | Enrolment: | Allocation: | Enrolment: | Study start date | Study start date | Study start date | Study start date | Study start date | Primary purpose: | Primary purpose: | Citation and official title                                      |
|---------------|-----------------------|-------------|------------|-------------|------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------------------------------------------------------|
| Sorafenib     | interventional (Phase I) | non-randomized, intervention | 12 participants | non-randomized, intervention | 12 participants | May 2007          | July 2010        | June 2011        | May 2007          | May 2011         | Treatment        | Treatment        | NCT00452218 (14636A) Sorafenib Study: Dosing in Patients With Pulmonary Arterial Hypertension (PAH) Gomberg-Maitland et al. (2010) Sorafenib Study: Dosing in Patients With Pulmonary Arterial Hypertension (PAH) (ARROW) |
| Nilotinib     | interventional (Phase I) | randomized, intervention | 23 participants | randomized, intervention | 23 participants | July 2010         | November 2014    | November 2014    | November 2014    | November 2014    | Treatment        | Treatment        | NCT01179737 (CAMN107X2201) Efficacy, Safety, Tolerability and Pharmacokinetics (PK) of Nilotinib (AMN107) in Pulmonary Arterial Hypertension (PAH) No corresponding publication A 24 Week, Randomized, Double Blind, Multicentre, Placebo-controlled Efficacy, Safety, Tolerability and PK Trial of Nilotinib (Tasigna®, AMN107) in Pulmonary Arterial Hypertension (PAH) |
| Selonsertib   | interventional (Phase II) | randomized, intervention | 151 participants | randomized, intervention | 151 participants | November 2014    | November 2014    | November 2014    | November 2014    | November 2014    | Treatment        | Treatment        | NCT02234141 (GS-US-357-1394) Selonsertib in Adults With Pulmonary Arterial Hypertension (ARROW) Rosenkrantz et al. (2017) A Phase 2, Dose-Ranging, Randomized, Double-Blind, Placebo-Controlled Study of GS-4997 in Subjects With Pulmonary Arterial Hypertension |
| Tacrolimus    | interventional (Phase II) | randomized, intervention | 23 participants | randomized, intervention | 23 participants | November 2014    | November 2014    | November 2014    | November 2014    | November 2014    | Treatment        | Treatment        | NCT01647945 (PAH-70522) FK506 (Tacrolimus) in Pulmonary Arterial Hypertension (Imatinib) in the Treatment of Severe Pulmonary Arterial Hypertension (TransformPAH) Spiekerkoetter et al. (2017) Single-Centre Randomized Controlled Phase II Study of Safety and Efficacy of FK-506 (Tacrolimus) in Pulmonary Arterial Hypertension |

**TABLE 2 (Continued)**

| Inhibitor | Design | Conditions | Identifier | Citation and official title |
|-----------|--------|------------|------------|----------------------------|
| Sorafenib | Type: interventional (Phase I) | Allocation: randomized, intervention | Enrolment: 23 participants | Study start date: June 2011 |
| Nilotinib | Type: interventional (Phase I) | Allocation: randomized, intervention | Enrolment: 23 participants | Study start date: July 2010 |
| Selonsertib | Type: interventional (Phase II) | Allocation: randomized, intervention | Enrolment: 151 participants | Study start date: November 2014 |
| Tacrolimus | Type: interventional (Phase II) | Allocation: randomized, intervention | Enrolment: 23 participants | Study start date: November 2014 |
Table 2 (Continued)

| Inhibitor     | Design                                      | Conditions                                                                 | Identifier         | Citation and official title |
|---------------|---------------------------------------------|-----------------------------------------------------------------------------|--------------------|------------------------------|
| Fasudil       | No clinical trial registered in the official databases from United States, Europe, or China | - PAH (by RHC) with mPAP ≥25 mm Hg, PCWP or LVEDP ≤12 mm Hg, PVR >5 mm Hg L^-1 min^-1  
- FEV1 ≥ 55% of normal  
- FEV1:FVC ratio ≥ 0.60  
- 6MWD ≥ 150 m and ≤ 450 m  
- WHO class III-IV  
- Pre-medication 2 or more specific standard PAH therapies for ≥8 consecutive weeks | NCT02587325 (PAH-001)  
ABI-009, an mTOR Inhibitor, for Patients With Severe Pulmonary Arterial Hypertension | Fukumoto et al. (2013), Jiang et al. (2014) |
| Nab-Sirolimus | Recruiting  
Type: interventional (Phase I)  
Estimated enrolment: 25 participants  
Intervention model: Single group assignment  
Masking: none (open label)  
Primary purpose: Treatment  
Study start date: April 2017 | - PAH (by PHC) with mPAP >25 mm Hg, PCWP ≤15 mm Hg and PVR >240 dynes cm^-5  
- 6MWD ≥ 150 m  
- WHO class III-IV; stable for at least 8 weeks  
- Expected survival of >6 months  
- Pre-medication with ERA or PDE5 inhibitors for at least 2 months  
- ALT or AST levels <3 times the upper limit of normal | NCT01083524 (DCA 20001)  
Dichloroacetate (DCA) for the Treatment of Pulmonary Arterial Hypertension | Michelakis et al. (2017)  
A Phase I, Open-Label, Two Centre Study to Evaluate Dichloroacetate (DCA) in Advanced Pulmonary Arterial Hypertension. |
| DCA           | Type: interventional (Phase I)  
Enrolment: 30 participants  
Allocation: non-randomized, intervention  
Model: parallel assignment  
Masking: none (open label)  
Primary purpose: Treatment  
Study start date: March 2010 | - PAH (by PHC) with mPAP >25 mm Hg, PCWP ≤15 mm Hg and PVR >240 dynes cm^-5  
- 6MWD ≥ 150 m  
- WHO class III-IV; stable for at least 8 weeks  
- Expected survival of >6 months  
- Pre-medication with ERA or PDE5 inhibitors for at least 2 months  
- ALT or AST levels <3 times the upper limit of normal | NCT01083524 (DCA 20001)  
Dichloroacetate (DCA) for the Treatment of Pulmonary Arterial Hypertension | Michelakis et al. (2017)  
A Phase I, Open-Label, Two Centre Study to Evaluate Dichloroacetate (DCA) in Advanced Pulmonary Arterial Hypertension. |

Note. All clinical trials listed in the ClinicalTrials.gov database investigating kinase inhibitors for the treatment of pulmonary hypertension are presented. In all cases, only participants (males or females) with an age of 18 years or older (in rare exceptions less than 70 years) and with a completed informed consent form were allowed to be enrolled in the studies.

decreased PTP expression and activity which contributes to elevated levels of PDGFRβ signalling (ten Freyhaus et al., 2011). But very recently, the inhibition of the Src homology 2 containing protein tyrosine phosphatase (PTP) 2 (Shp2) by Phps-1, a highly specific Shp2 inhibitor, improved several cardio-pulmonary disease parameters in MCT-induced PAH in rats, demonstrating that, in principle, PTPs might potentially be new targets for the treatment of PAH (Cheng et al., 2018).

Another yet unknown phenomenon in the field of PAH appeared recently: For certain growth/cytokine factor receptors, soluble protein variants produced either by proteolytic cleavage or by alternative splicing showed abnormal levels in plasma from PAH patients (Tiede et al., 2015). Whether those signatures, for example, for sFlt-1 (sVEGF), can be considered as PAH-specific biomarkers or effectors of disease progression still needs to be determined in larger cohorts with exclusion of other (co-)morbidities.

The challenges of a therapeutic exploitation of kinase inhibitors are the translation of successfully tested drugs from preclinical animal models into the setting of human PH and the prevention of unwanted side effects. Investigations in multiple experimental models are required to overcome the first hurdle, but still they do not fully resemble the pathological phenotype of the pulmonary vasculature and of the right ventricle of PH patients. In addition, it should be noted that PH rather consists of a group of heterogeneous subtypes arising from several different causes with complex clinical symptoms, rather than being a well-characterized disease. This aspect could potentially be addressed with the help of personalized medicine which would enable physicians to classify the patients for distinct treatment options prior drug administration (Figure 1). Personalized medicine is one of the new concepts in clinical drug development in general and also for pulmonary hypertension which is not only restricted to kinase inhibitors but also applies for other compounds as well. First, data from basic research and clinical examinations are collected to improve the understanding of the disease. At the bench-side, in vitro as well as in vivo studies using experimental models are employed to deeply
analyse the pathobiology and the disease mechanisms of PH (research data). At the same level, distinct patient subgroups (coloured symbols) with similar clinical features are identified, further reflecting the interpersonal differences (patient heterogeneity) at the bed side. Those individual patient data (clinical data) might only show a partial match (overlapping areas) with the hypothesis generated at the bench side. To fully characterize the various patient cohorts not only on the clinical but also on the molecular level, large scale "-omic" approaches can be used to obtain comprehensive individual data sets at multiple biological and clinical levels including imaging, function, metabolomics, proteomics, biomarker, epigenetics, kinomics, and genomics (technologies). These newly available technologies in combination with routine clinical inspections now offer an in-depth phenotyping of all individual patients (phenotyping). Through this approach, yet unknown pathobiological features of PH as well as of cellular signalling events can be discovered which discriminate the diseased status from healthy conditions. With respect to signalling pathways and intracellular mechanisms involved in disease onset and progression, protein kinases can be validated as important players and as possible drug targets in vitro. This facilitates the selection of compounds of interest, for example, kinase inhibitors, to be tested in preclinical studies in PH models in vivo. Drug development then includes screening of a compound library and the identification of a lead structure finally resulting in a new chemical molecule with the best performance in activity-based high throughput assays. In PH, many anti-cancer drugs have been analysed for their efficacy to inhibit hyper-proliferation of pulmonary vascular cells and/or to directly interfere with the right ventricular remodelling process. Experimental PH models are employed to demonstrate the kinase inhibitors’ mode of action and any signs of systemic and/or cardiac toxicity. For the latter case, long-term treatment studies in animals could reveal unknown effects (including adverse events) in the lung and the heart. In the final step, clinical studies are conducted to analyse the mode of action, tolerability, and possible signs of (hidden) toxicity in human PH. In phase II and III studies, drug responses will be detectable on the individual basis, and corresponding biomaterial sampling will allow a deeper investigation on the cellular level. Distinct degrees of responsiveness to a given drug for a patient subgroup (coloured symbols) might be observed, which can be linked to activity or expression profiles, that is, of biomarkers, or even a particular genetic background. As the costs for DNA

FIGURE 1  Targets of kinase inhibitors for the treatment of pulmonary hypertension. Kinases for targeted inhibition are depicted in red while other intracellular signalling proteins are coloured in grey. Membrane receptors are highlighted in beige. Coloured connections reflect activating protein–protein interaction (green) or blockage of protein function (red). Interference with kinases activity in the pulmonary vasculature by the depicted inhibitors leads to a reduction in proliferation, inflammation, and vasoconstriction which subsequently promotes the reversal of the vascular remodelling process. In the right ventricle, anti-hypertrophic and anti-fibrotic effects are observed resulting in improved cardiac function.
sequencing have been dramatically decreasing, whole-exome sequencing is now affordable and can provide information about possible SNPs or crucial mutations which influence the therapeutic outcome of the individual patients. This hypothesis is a central aspect of a relatively novel field of research named pharmaco-genomics which combines pharmacology and genomics to study how individual genes affect a patient’s response to a certain drug. Thus, many critical determinants of successful medication like doses, safety, efficacy, can be linked to a person’s genetic background which allows improved and personalized medicine. For further details see text.

13.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander, Christopoulos, et al., 2017; Alexander, Fabbro, et al., 2017a, 2017b).

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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