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Review Article

Bone morphogenetic protein receptors: Structure, function and targeting by selective small molecule kinase inhibitors

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ABSTRACT

Bone morphogenetic proteins (BMPs) are secreted cytokines that control the fate and function of many different cell types. They exert their cellular responses via heteromeric complexes of specific BMP type I and type II serine/threonine kinase receptors, e.g. BMPRIA and BMPRII. Three type II and four type I receptors, also termed activin receptor-like kinases (ALKs), have been identified. The constitutively active type II kinase phosphorylates the type I receptor, which upon activation initiates intracellular signaling by phosphorylating SMAD effectors. Auxiliary cell surface receptors without intrinsic enzymatic motifs, such as Endoglin and Repulsive guidance molecules (RGM), can fine-tune signaling by regulating the interaction of the BMP ligands with the BMPRs. The functional annotation of the BMPR encoding genes has helped to understand underlying mechanisms of diseases in which these genes are mutated. Loss of function mutations in *BMPRII*, *Endoglin* or *RGMc* are causally linked to pulmonary arterial hypertension, hereditary hemorrhagic telangiectasia and juvenile hemochromatosis, respectively. In contrast, gain of function mutations in *ACVR1*, encoding ALK2, are linked to Fibrodysplasia ossificans progressiva and diffuse intrinsic pontine glioma. Here, we discuss BMPR identification, structure and function in health and disease. Moreover, we highlight the therapeutic promise of small chemical compounds that act as selective BMPR kinase inhibitors to normalize overactive BMPR signaling.

1. Introduction

A pioneering study by Urist indicated that demineralized bone extracts contained bone inducing factors, which he termed bone morphogenetic proteins [1]. BMPs were thereafter isolated to homogeneity from demineralized bone extracts using a tedious ectopic bone formation assay in rodents [2]. The subsequent amino acid sequencing of peptide fragments allowed for PCR-based cloning of cDNAs encoding BMPs. The predicted primary amino acid sequence revealed that they are related to transforming growth factor-β (TGF-β) and activin [3].

These proteins are part of the so-called TGF-β family and are synthesized and secreted as large precursor proteins that are proteolytically processed, releasing their bioactive region located at the carboxy-terminus. The biological functions ascribed to BMPs have greatly diversified since their discovery. Like other TGF-β family members, they are multifunctional proteins that act in a highly context-dependent manner [4], BMPs act as morphogens in early development, steer organ formation and mediate tissue homeostasis. It comes as no surprise therefore, that perturbations of the functions of BMPs lead to severe developmental disorders and a large spectrum of diverse human

Abbreviations: ACVR, activin A receptor type, gene encoding activin receptor; ActR, activin receptor; ALK, activin receptor-like kinase; AON, anti-sense oligonucleotide; BMP, bone morphogenetic protein; BMPR, BMP receptor; Co-SMAD, common mediator SMAD; DIPG, diffuse intrinsic pontine glioma; DM, Dorsomorphin; FKBP12, FK506 binding protein 12; FK506, also known as Tracolimus or fujimycin; GS-domain, glycine-serine rich domain; GTPase, guanosine triphosphate hydrolytic enzyme; FOP, Fibrodysplasia ossificans progressiva; HHT, hereditary hemorrhagic telangiectasia; I-SMAD, inhibitory SMAD; miRNA, micro RNA; RGM, repulsive guidance molecule; R-SMAD, receptor regulated SMAD; siRNA, small interference RNA; SMAD, Sma and Mad related protein; SMURF, SMAD ubiquitin regulatory factor; TGF-β, transforming growth factor-β; TβR, TGF-β receptor; VEGF, vascular endothelial growth factor

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diseases [5–7].

Individual members of the TGF-β family share a high sequence similarity with each other and are highly conserved in evolution. It was in line with expectations that they would signal via structurally related receptors. After the expression cloning of the cDNA encoding the activin type II receptor (ActR2) [8], multiple labs initiated cloning strategies to identify related receptors. The predicted primary amino acid sequence revealed that these receptors contain an intracellular serine/threonine kinase domain. Based upon the kinase domain, degenerate PCR primers were used to clone related receptors [9,10]. The identification of transmembrane BMP receptors was performed by affinity labeling experiments using radiolabeled BMP ligands, on cells overexpressing ActR2-related proteins [11–15]. The receptors can be grouped in two distinct subfamilies, so-called type I (also termed activin receptor-like kinases) and type II receptors.

In a series of seminal manuscripts by the Massagué laboratory, TGF-β was shown to signal via heteromeric complexes of TGF-β type I and TGF-β type II receptors (TβRI and TβRII), in which TβRI acts as a substrate of TβRII [16,17]. BMPs signal in a similar manner with the type I and type II receptors acting sequentially, with the type I specifying the intracellular response within the BMPR heteromeric complex. Three type II receptors have been identified for BMPs; BMPRII (*BMPR2*) that is unique for BMPs, and activin type II receptors ActRIIA (*ACVR2A*) and ActRIIB (*ACVR2B*) that are shared with activins. Four type I receptors, also termed ALK1 (*ACVRL1*), ALK2 (*ACVR1*), ALK3 (*BMPRIA*) and ALK6 (*BMPRIB*) were found in the mammalian genome [18]. Upon BMP-induced heteromeric complex formation, the type II constitutively active kinase transphosphorylates the type I receptor kinase. The activated type I receptor then initiates intracellular signaling by phosphorylating downstream effector molecules, in which the SMAD proteins play a key role [19,20]. Distinct sets of so-called receptor-regulated (R-)SMADs are recruited by either BMP type Is or TβRI and activin type I receptor ALK4 for activation. Phosphorylated R-SMADs form heteromeric complexes with the common mediator SMAD4 [21,22], and accumulate in the nucleus where they bind DNA and participate in gene transcriptional responses [23–25]. The pleiotropic and relevant action of BMPs is reflected in the extensive regulation of the expression, stability, localization and function of BMPRs and SMADs, in response to extracellular and intracellular cues. Mutations in specific BMP receptors are linked to developmental disorders and a broad range of postnatal diseases, including musculoskeletal, cardiovascular diseases, and many cancers [18]. Fortunately, the receptors with their ligand binding domains and intracellular enzymatic activity are eminently druggable targets. Antibodies or ligand traps that interfere with BMP-receptor binding, and selective BMPR kinase inhibitors are both examples that show clinical promise [26–29]. In this review, we present our current understanding regarding BMPR structure and function in health and diseases. In particular, we will focus on human diseases in which certain BMPR encoding genes are corrupted. We also discuss the identification of selective small molecule BMP receptor kinase inhibitors. Due to the increasing number of BMPR kinase inhibitors reported and their recent inclusion in human clinical trials, we will focus on these molecules, while other therapeutic agents, such as BMP ligand traps, are only briefly cited.

2. BMP receptor signaling

BMPs are homo- or heterodimers that interact with selective heteromeric complexes of two type I and two type II receptors. BMPs bind weakly to the type I or type II receptor, but the affinity is promoted when BMP engages the type I-type II heteromeric complex [18]. The overall architecture of the extracellular domains is highly conserved across receptor complexes with the tetramer displaying a 2-fold symmetry axis (Fig. 1A–B). The type II domains form an intermolecular disulfide bridge while the type I receptors contain specific 'binding loops' at the tetramer interface (Fig. 1C). Subtle sequence changes in this loop can modulate its conformation and thus the affinity of the type I- type II interaction. The interaction of the tetramer with the BMP ligands is modulated by specific 'hot spots' on the binding surface of the tetramer that allow for alterations of specificity between ligand and receptor complex [30,31].

Different BMP dimers bind with distinct affinity to different heteromeric complexes [32]. For example, focusing on different BMP subgroups and interaction with type I receptors, BMP9 shows highest affinity for ALK1 and binds weakly to ALK2 [33]; BMP10 preferentially binds ALK1, over ALK3 and ALK6; BMP5, BMP6 and BMP7 signal via ALK2, although BMP6 can also bind ALK3 and ALK6; BMP2 and BMP4 interact most strongly with ALK3 and ALK6 [11,34] (see excellent reviews [32,35–38]). Furthermore, recent publications have suggested the existence of endogenous BMP heterodimers in mammalian organisms (i.e., BMP9/BMP10 [39], BMP7/BMP2, BMP7/BMP4 [40]). As reported for artificially generated heterodimers, heterocomplexes may exhibit higher activity than homodimers in some cases [41,42]. Whether these molecules are present in all tissues and physiological conditions remains an area of extensive study.

BMPs can bind to heteromeric complexes consisting of different type I receptors. Such complexes, with different type I receptors, may show greater signaling activity [43,44]. Ligand access to the signaling receptors is regulated by binding to extracellular matrix (ECM) components and soluble ligand binding proteins [45]. Auxiliary accessory receptors or "co-receptors" regulate the interaction of BMP ligands to the signaling receptors [46,47]. Examples of such co-receptors include Endoglin, which is a transmembrane receptor with a short intracellular domains that lacks an enzymatic motif [48], and the Repulsive guidance molecules (RGMs) A, B and C (also termed hemojuvelin) that are linked to the plasma membrane via a glycosylphosphatidylinositol (GPI)-anchor [49]. These co-receptors can present or mask BMP ligands to or from signaling receptors [47]. By the action of specific proteases, they can be shed from the plasma membrane, and thereby limit BMP bioavailability [50]. Together the ECM, soluble ligands and co-receptors allow for a careful spatio-temporal control of BMP's as morphogens during development and in the adult to maintain tissue and organ homeostasis.

While type I and type II receptors form two distinct subgroups based on their sequence similarity, both receptor types share a similar domain structure: a cysteine-rich extracellular domain that mediates interaction with the BMP ligand, a single transmembrane region, and an intracellular part that is nearly entirely comprised of a serine/threonine kinase domain [18]. Key differences between the intracellular domains of type I and type II receptors are that type II's have a small kinase insert, and that type I's have a juxtamembrane region rich in serine and glycine residues (GS-domain) (Fig. 1D). The type II phosphorylates specific serine and threonine residues in the GS-domain, leading to type I receptor activation allowing for SMAD phosphorylation at two carboxy-terminal serine residues. The leucine-proline residue motif in the GS-domain is a recognition site for the peptidyl-prolyl cis-trans isomerase FKBP12. By shielding the serine and threonine residues from phosphorylation by the type II kinase, FKBP12 sets a threshold for (and negatively regulates) type I receptor activation [51] (Fig. 1E).

Upon type I receptor phosphorylation by the type II receptor kinase the signal is transmitted into the cell by phosphorylating R-SMAD effector proteins [19,20] that, in concert with SMAD4 [22], regulate transcriptional responses [23]. Studies have shown that a COOH-terminal sequence between residues 482–491 in the kinase domain of the type I receptor, the so-called NANDOR BOX, is critical for the activation of the receptor [52]. SMADs interact only weakly with DNA and there is thus a need for SMADs to cooperate with other DNA binding transcription factors, coactivators and corepressors [53]. An extensive overview of mechanisms and regulators that control SMAD function is beyond the scope of this review and therefore we only briefly discuss a few examples. The R-SMAD-SMAD4 complex formation, localization, stability and interaction with other co-factors,

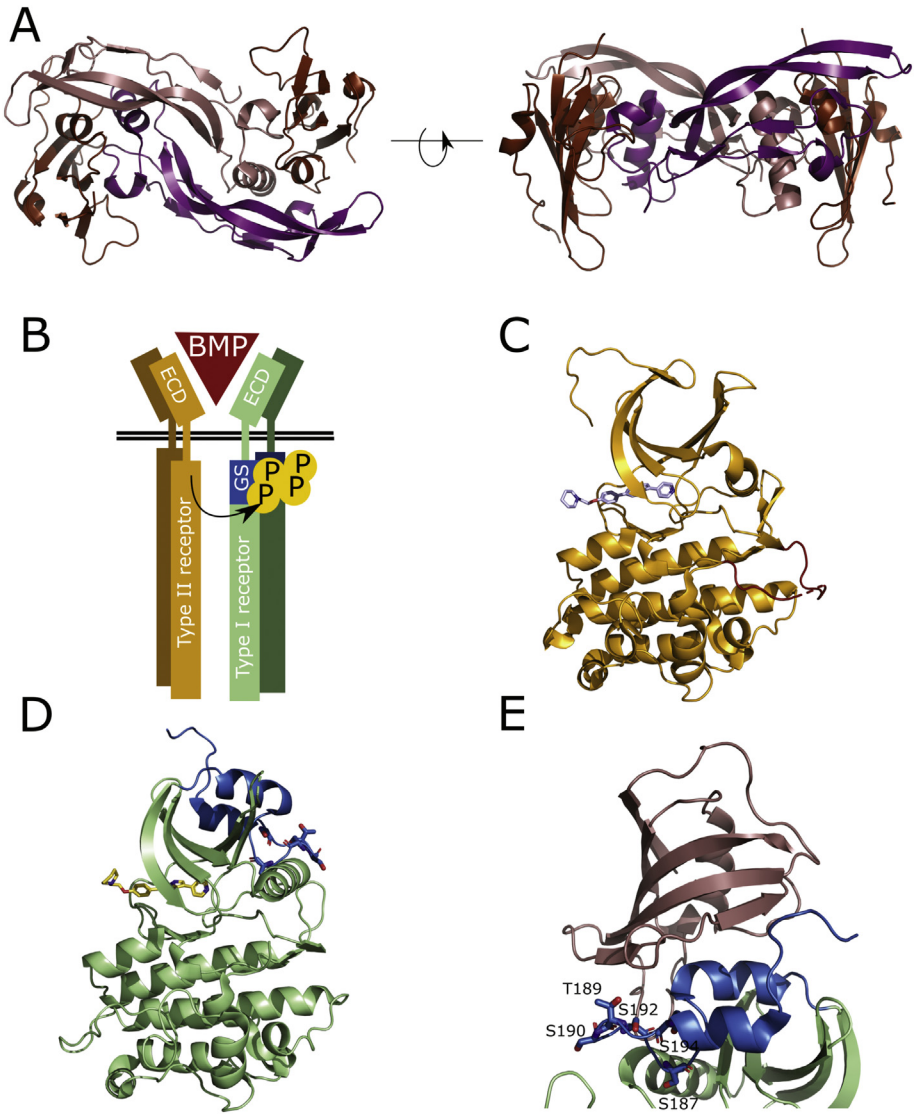


Fig. 1. Structural details of BMP type I and type II receptors. A) The arrangement of the type I and type II receptors, in this case BMPR2 (purple) and BMPR1A (dark red) showing the view from above (left) and rotated by 90 to show the cross-sectional view orientated with the membrane beneath it (right). B) A cartoon schematic of the assembly and function of the type I (kinase domain: aqua. GS domain: blue) and type II (kinase domain: orange) receptors in the membrane. C) Type II receptor ACVR2A (orange) showing type II loop insertion (red). D) Intracellular domain of the type I receptor (ALK2 pdb:4C02) showing the kinase domain (aqua) and the GS loop (blue). E) Interface between ALK2 (kinase domain in green. GS loop in blue) and the inhibitory protein FKBP12 (maroon) showing key Ser and Thr residues within ALK2 which when phosphorylated inhibit FKBP12 binding (pdb:4C02).

thereby regulating its function, are highly regulated by post-translational modifications, including phosphorylation, acetylation, ubiquitylation, SUMOylation, PARrylation, etc [54]. The inhibitory SMADs (I-SMADs; i.e. SMAD6 and SMAD7) are strongly induced by BMPs and limit BMPR/SMAD signaling by regulating the intensity and duration of the BMP/SMAD response. Moreover, I-SMADs expression and stability are regulated by other extracellular cues. The I-SMADs exert their antagonistic effects by multiple mechanisms, including recruitment of SMURF1/2 E3 ubiquitin ligases [55], which mediate ubiquitination and thereby facilitate proteasomal and lysosomal degradation. This is a reversible process; specific deubiquitinating enzymes can remove the ubiquitin (chains) of BMP signaling mediators or regulators [56]. All these control mechanisms allow for fine-tuning and integration of BMPR/SMAD signaling with other signaling pathways.

The BMP/SMAD pathway appears simple: BMP receptors become ligand activated and SMADs relay the receptor activation signal from the plasma membrane into the nucleus. There are no intracellular enzymatic amplification steps involved, as is the case for tyrosine kinase receptors [57]. Therefore, in line with its morphogen action the amount of active BMP ligand determines the level of activated SMAD [58] (Fig. 2). Activated BMPRIs, i.e. ALK1, 2, 3 and 6 induce the phosphorylation of SMAD1, SMAD5 and SMAD8. The TβRI/ALK5 and activin type I receptor ALK4, in contrast, mediate the phosphorylation of SMAD2 and SMAD3 [59]. This is in line with the central dogma for TGF-β family receptor activation in which the type I receptor determines the signaling specificity [60], and that BMPs as a group share common effects that are different from TGF-β/activin signals. However, type II receptors (and/or auxiliary receptors) may also determine the signaling response by recruiting specific molecules into the receptor complex [61]. Besides the canonical SMAD pathway, BMP receptors can also initiate non-SMAD signaling pathways, which includes activation of extracellular signal- regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) MAP-kinases and small GTPases such as Rac and Rho [62]. Importantly, TGF-β/Activin and BMP signaling pathways usually counterbalance each other at different levels, and both pathways crosstalk with other signal transduction cascades, to regulate essential physiological processes. Disturbances in the BMPR signaling axis, for example by means of genetic mutations in BMPRs that affect their function, have been identified as the underlying cause of several human disorders.

3. BMPR mutations in disease

Genetic lesions in BMPRs are linked to musculoskeletal and cardiovascular disorders, and identified in many cancers (Table 1), showing the importance of BMPR signaling for normal development and physiology [18,63]. Disease causing mutations identified in BMPRs either enhance or disrupt BMP signaling. In the subsequent paragraphs we will

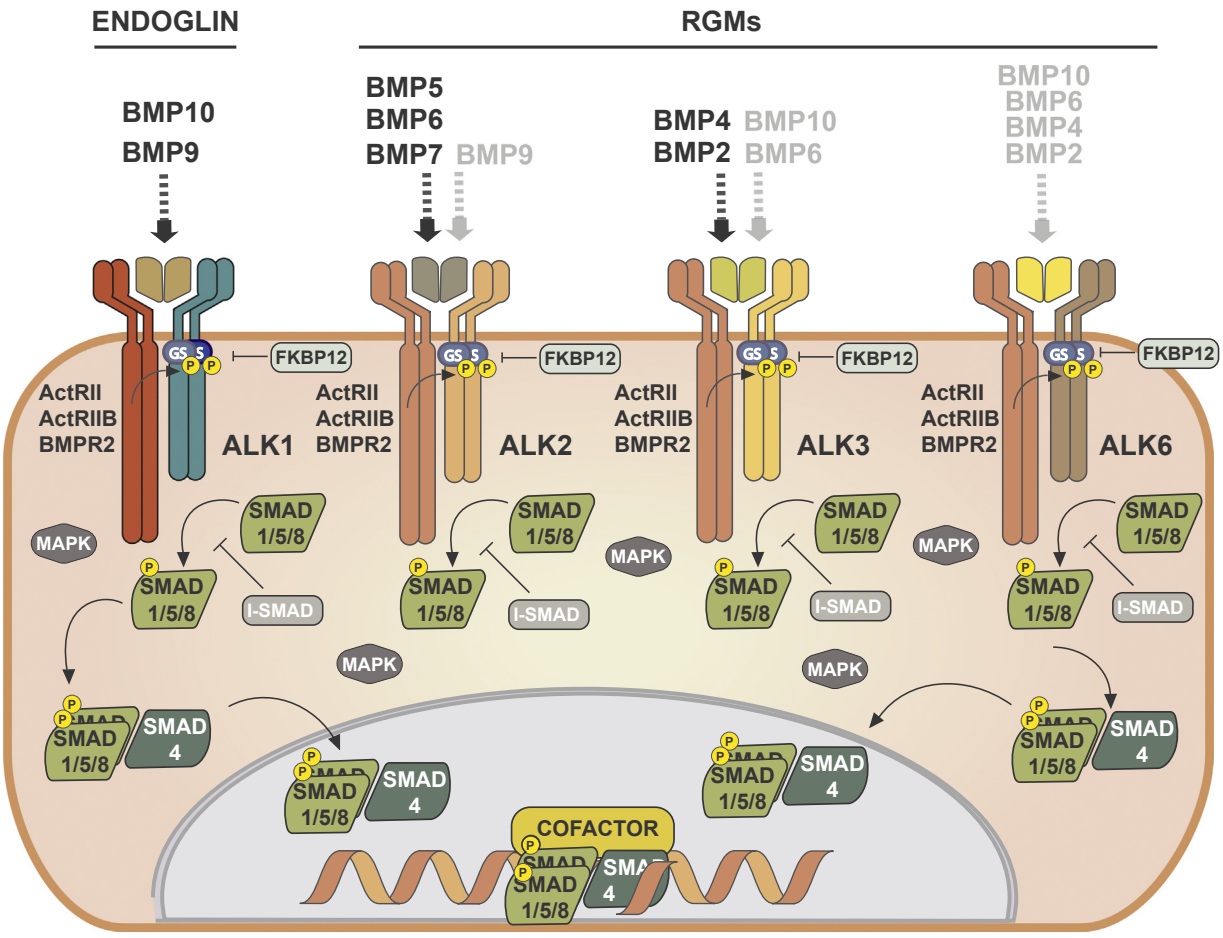


Fig. 2. BMPR signaling. BMPs exert their cellular responses via BMP type I and type II receptors. Three BMP type II receptor have been identified, i.e. BMPRII, ActRII and ActRIIB, and four BMP type I receptors, i.e. ALK1 (ACVRL1), ALK2 (ACVR1), ALK3 (BMPRIA), ALK6 (BMPRIB). Soluble BMP ligands preferentially bind specific type I receptors (bold ligands), although alternative low affinity interactions have been reported (light grey ligands). Upon BMP-induced heteromeric complex formation, the constitutively active type II receptor phosphorylates and activates the type I receptor. Thereafter, the signal is transduced into the cell by action of ALK1/2/3/6 that phosphorylates R-SMAD1, 5 and -8. Activated R-SMADS form complexes with SMAD4, which act as DNA sequence specific transcription factor complexes that regulate gene transcriptional responses. Inhibitory SMADs (I-SMADs) act to antagonize signal transducing R-SMADs. In addition to canonical SMAD pathway, BMPR initiate non-SMAD (MAPK) signaling responses.

Table 1
BMPR genetic mutations in human disease. HHT: hereditary hemorrhagic telangiectasia; PAH: pulmonary arterial hypertension; FOP: Fibrodysplasia ossificans progressiva; DIPG: diffuse intrinsic pontine glioma; AVSD: atrioventricular septum defect; JPS: juvenile polyposis syndrome; CHD: congenital heart disease; ASD atrial septal defects; AMDD: acromesomelic dysplasia Demirhan type; AMDHT: acromesomelic dysplasia Hunter-Thompson type; BDA1D: brachydactyly type A1D; BDA2: brachydactyly type A2; RGMc/HJV: repulsive guidance molecules type c/ hemojuvelin; JV: juvenile hemochromatosis.

BMP receptor	Disease	Mutation	Reference(s)
ACVRL1 (ALK1)	HHT2	No specific hotspot. 75% intracellular. Loss of function mutations resulting in haploinsufficiency.	[64]
	PAH, in the absence of diagnosed HHT	Loss of function mutations in GS domain or NANDOR box	[65]
ACVR1 (ALK2)	FOP	R206H in 95% of patients. Gain of function mutation.	[67]
	DIPG	Seven gain of function mutations in GS domain.	[72,73]
		R206H in common with FOP, G238V unique for DIPG	[74]
	AVSD	L343P. Dominant negative receptor	[79]
BMPR1A (ALK3)	JPS	No specific hotspot but R443C most frequent. Loss of function mutations.	[84]
	JPS with CHD (Ebstein's anomaly, AVSD)	R443H. Dominant negative receptor.	[85]
	ASD	R478H, D429V, P481S, R406L. Mutations impair signaling.	[86]
BMPR1B (ALK6)	AMDD	del359-366. Loss of function mutation.	[88]
	AMDHT	M397R. Reduced kinase activity.	[89]
	BDA1D	K325N. Reduced kinase activity.	[90]
	BDA2	I200K, R486W, R486Q. Inhibit BMP signaling.	[91]
	PAH	S106N, F392L. Gain of function mutation.	[92]
BMPRII	PAH	No specific hotspot. Loss of function mutations.	[163]
ENG	HHT1	No specific hotspot. Most extracellular. Loss of function mutations.	[100]
RGMc/HJV	JH	No specific hotspot but G320V most frequent. Inhibit BMP signaling.	[106]

describe disorders to which each of the BMPRs has been associated.

3.1. ALK1

Hereditary hemorrhagic telangiectasia 2 (HHT) 2 or Rendu-Osler-Weber disease is linked to the presence of mutations in *ACVRL1* (encoding ALK1) [64]. HHT is an autosomal dominant disease characterized by vascular dysplasia like mucocutaneous and visceral telangiectasia and arteriovenous malformations. Telangiectasia is prone to rupture, resulting in the most frequent symptom in HHT patients: epistaxis. To date, over 185 pathogenic mutations have been identified in *ACVRL1*, resulting in either reduced levels of ALK-1 or a nonfunctional mutant protein. *ACVRL1* mutations in the GS domain or the NANDOR box have been uniquely linked to pulmonary arterial hypertension (PAH) in the absence of diagnosed HHT [65]. Recently, 3 new mutations were identified in the kinase domain of *ACVRL1* in iPAH and hPAH patients without HHT [66].

3.2. ALK2

The gain-of-function mutation R206H in *ACVR1* (encoding ALK2), located in the intracellular juxta-membrane GS domain of ALK2, is linked to 95% of the patients developing Fibrodysplasia ossificans progressiva (FOP) [67] while the minority of patients carry other gain of function mutations in residues located in other parts of the cytosolic domain of ALK2 [68]. FOP is a rare devastating autosomal-dominant disorder characterized by episodic heterotopic ossification (HO) of muscle, fascia, ligaments, and tendons. All mutant ALK2 receptors associated with FOP are able to bind and signal upon binding of activin A [68,69], and signaling is not suppressed by the negative regulator FKBP12 [70,71], thereby driving progressive extra-skeletal bone formation and causing patients to be wheelchair bound by their 30s – 40s. Moreover, *ACVR1* is the 3rd most commonly mutated gene found in approximately 25% of patients with the rare childhood brainstem tumor diffuse intrinsic pontine glioma (DIPG; [72–74]). Seven somatic point mutations have been identified for DIPG, that cluster either in the GS-domain or the kinase domain of ALK2 [75]. Six of these mutations are similar to those found in FOP, including the FOP classical R206H mutation [76]. The G238V mutation in ALK2 is exclusively found in DIPG. All 7 mutations lead to a gain of function of the receptor resulting in an increase in BMP signaling within the tumors and subsequent susceptibility to drugs targeting ALK2 [77]. Interestingly, although FOP patients present identical mutations in ALK2, there is no predisposition to the development of DIPG in these patients. The co-segregation of H3.1K27 observed in DIPG might explain the difference [78].

In addition to mutations leading to receptor activation, loss of function mutations in *ACVR1* have been described in human cardiovascular disorders. Atrioventricular septum defect (AVSD) is linked to a L343P mutation in *ACVR1* [79]. L343P is located within the kinase domain of ALK2 and abolishes its activity, rendering the mutant receptor dominant negative. When an ALK2-L343P plasmid was injected into the developing zebrafish embryo, formation of the AV canal was hampered. Furthermore, a H286N substitution in ALK2 was identified in a person with Down syndrome and a patient with an atrial septal defect [80]. This mutation is within the ATP binding pocket of the ALK2 kinase domain and reduces its activity. Interestingly, right ventricular dilation and signs of cardiomyopathy have been reported in FOP patients carrying the R206H mutation [81].

3.3. ALK3

Mutations in *BMPRIA* (encoding ALK3) have been associated with the occurrence of the autosomal dominant inherited disorder juvenile polyposis syndrome (JPS [82]). JPS patients with mutations in *BMPRIA* have a 30% change of developing colon cancer [83]. Missense mutations are found in all regions of *BMPRIA* except in the transmembrane domain [84] and impair signaling either by reducing kinase activity and/or reducing localization at the cell membrane. JPS is often linked to congenital heart disease (CHD). The R443H mutation in BMPRIA is associated with the development of AVSD and Ebstein's anomaly [85]. Furthermore, CHDs, including AVSDs, are linked to deletions of *BMPRIA* in patients carrying the 10q22q23 microdeletion syndrome [86]. The homozygous missense variant R406L, located in the kinase domain of ALK3, causes both severe skeletal malformations and atrial septum defects [87]. Interestingly, while ALK3-R406L shows only a modest to normal BMP-induced SMAD activation, non-SMAD signaling was severely impaired.

3.4. ALK6

Acromesomelic dysplasias (AMD) are rare hereditary skeletal disorders linked to loss of function mutations in *BMPR1B* (ALK6) and characterized by short stature, very short limbs and hand/foot malformations. The severity of limb abnormalities increases from proximal to distal and profoundly affects hands and feet showing only brachydactyly and/or rudimentary fingers. AMD Demirhan type is an autosomal recessive disorder linked to an 8 bp deletion in *BMPR1B* [88], while AMD Hunter-Thompson type is caused by a missense mutation (M397R) within the receptor kinase domain [89].

Brachydactyly is an autosomal dominant disorder characterized by shortening of the digits due to abnormal development of the phalanges and/or the metacarpals. Brachydactyly type A1 (BDA1) is primarily characterized by hypoplasia/aplasia of the middle phalanges of digits 2–5. In brachydactyly type A2 (BDA2) shortening of the middle phalanges is confined to the index finger and the second toe, all other digits being more or less normal. Both BDA1 [90] and BDA2 [91] are related to mutations in the kinase domain or GS domain of ALK6 resulting in decreased kinase activity and impaired cartilage formation.

Finally, two gain of function mutations were found in *BMPR1B* in pulmonary arterial hypertension (PAH) patients. S106N is located outside of any functional domain of ALK6 while F392L is located in the kinase domain of the receptor [92].

3.5. BMPRII

To date, over 350 pathogenic *BMPR2* gene mutations have been reported that can cause PAH, although penetrance is only approximately 14% [66,93]. PAH is a rare and severe pulmonary vascular disorder characterized by abnormally high pulmonary arterial pressure, resulting in progressive occlusion of the vascular lumen due to an increase in endothelial and smooth muscle cell proliferation. Ultimately, PAH patients develop fatal right heart failure [94]. In > 70% of all hereditary PAH patients, mutations in *BMPR2* have been identified that compromise its function. There is no clear mutation hotspot; mutations are located in the ligand binding domain, kinase domain and the long cytoplasmic tail of the receptor. Half of the mutations disrupt the assembly of BMPRII, thereby reducing the amount of protein in the cell, while others mutations prevent BMPRII from reaching the cell surface, or reduce ligand binding or kinase activity [95–97].

BMPR2 mutations have also been linked to different forms of cancer. Since germline mutations in *BMPR1A* are linked to colorectal cancer (CRC) [98], disturbed BMP signaling was investigated in sporadic CRC. Interestingly, Kodach and co-workers demonstrated that *BMPR2* was mutated in microsatellite unstable CRCs, resulting in reduced levels of BMPR2 [99].

3.6. Endoglin

While mutations in *ACVRL1* cause HHT2, germline mutations in *ENG*, encoding Endoglin, are linked to HHT1 [100], resulting in haploinsufficiency of Endoglin [101]. To date, over 500 pathogenic mutations in *ENG* have been registered [102] (https://arup.utah.edu/

database/ENG/ENG_welcome.php).Although there are no common mutation hotspots, many mutations are located in the DNA sequence encoding for the extracellular region of Endoglin, the largest part of the co-receptor [103].

3.7. RGM

Three members of the family of repulsive guidance molecules, RGMa [104], RGMb (Dragon) [105] and RGMc [106] (hemojuvelin or HJV) function as co-receptors to enhance BMP signaling. Mutations in *RGMc/HJV* are linked to the rare genetic disorder juvenile hemochromatosis. Juvenile hemochromatosis is characterized by the accumulation of iron in various organs of the body resulting in multiorgan dysfunction such as cirrhosis, cardiomyopathy, and diabetes mellitus before the age of 30 [107]. The disease-associated mutations in *RGMc/HJV* account for > 90% of the known cases [108]. To date there are 43 mutations reported in *RGMc/HJV* of which G320V is the most frequent one [109].

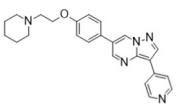
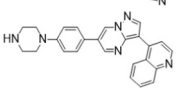
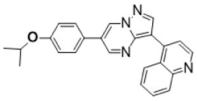
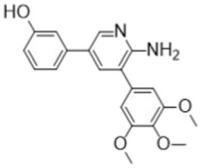
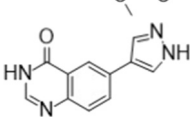
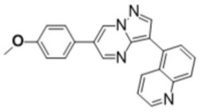
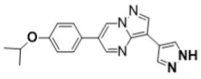
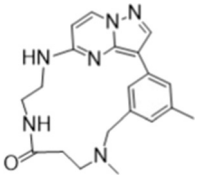
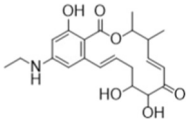
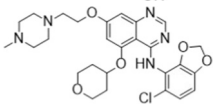
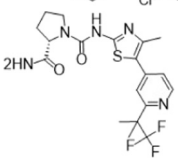
4. Pharmacological agents to normalize overactive BMPR signaling

As aforementioned, BMPs are pleiotropic molecules controlling key physiological functions in different tissues. Although it might be clinically desirable to normalize exacerbated BMP activity in certain pathological conditions, this should be achieved in a very specific manner, for example, by restricting the delivery of an inhibitor to a particular tissue, or by identifying and targeting specific disease-related aberrant mechanisms downstream of BMPR. In this sense, during the last decades efforts have been made to develop potential therapeutic agents targeting different levels within the BMP signaling cascade. Those include molecules which compromise ligand bioavailability (such as natural soluble antagonists, specific antibodies and ligand traps), inhibitors of BMPR expression (siRNAs [110], anti-sense oligonucleotides [111], miRNAs [112], transcription inhibitors [113]), molecules that prevent BMPR activation (including receptor antibodies [114], kinase inhibitors) and intracellular inhibitors (for example, SMAD inhibitors [115]). Moreover, a number of compounds have been reported to inhibit signaling downstream of BMPR, although some of them were not originally conceived as BMPR antagonists (i.e., BYL719, Palovarotene, rapamycin, Saracatinib, see below). Due to the recent advances in the development of more specific low-molecular-weight molecules, some of which are currently being evaluated in clinical trial programs, we will mainly focus on small molecules to inhibit BMPR kinase activation in this review (Table 2).

The high structural similarity displayed between the ATP-binding pocket in cellular kinases compromises the selectivity of ATP competitive kinase inhibitors [116]. Most of the reported molecules are type I inhibitors, which displace ATP from the catalytic pocket of the kinase domain. The first potent BMP type I receptor kinase inhibitor named Dorsomorphin (DM), was identified out of 7500 compounds using an in vivo zebrafish dorsalization phenotypic screen. Dorsoventral patterning in the fish embryo is organized by a gradient of ventralizing BMPs and antagonists arising from opposite areas [117]. BMP antagonists (i.e., Noggin, Chordin and Follistatin) therefore promote embryonic dorsalization, which was phenocopied by the administration of DM. DM effectively suppressed BMP signaling downstream of ALK2, ALK3 and ALK6 in mammalian cells. However, DM was in fact previously characterized under the name Compound C as an inhibitor for 5′ AMP-activated protein kinase (AMPK) among many kinases (Supplementary Fig. 1A) [118]. DM is very similar to other VEGFR kinase inhibitors [119], therefore its use may lead to detrimental effects on the vasculature. DM's IC_{50} to inhibit ALK-induced SMAD1/5/8 phosphorylation was calculated (IC_{50} = 0.5 μM). A structure-activity relationship (SAR) study based on DM led to the discovery of substituted pyrazolo[1,5-*a*] pyrimidine derivatives, in which LDN-193189 was identified as a novel molecule with improved pharmacokinetic properties and an IC_{50} value for BMP4-induced SMAD1/5/8 phosphorylation inhibition in the nanomolar range (Fig. 3A) [120]. LDN-193189 was subsequently used to demonstrate the contribution of over-activated BMPR signaling to the development of FOP. Using a mouse model of FOP, characterized by the combination of adenoviral expression of a constitutively active version of ALK2 (Q207D) and cardiotoxin-mediated injury, Yu et al., showed how intraperitoneal administration of LDN-193189 (3 mg/kg) was able to partially prevent heterotopic ossification [121], without inducing osteopenia or bone fractures. Subsequently, LDN-193189 was applied in a number of studies to show the benefit of preventing BMPR activation in chronic kidney disease [122], vascular calcification [123] and atherosclerosis [124], prostate cancer [125] and DIPG [77], among others. One should note, however, that LDN-193189 potently inhibits, in addition to BMP type I receptor kinases, other cellular kinases [126], including VEGFR. Therefore, a second in vivo zebrafish dorsalization assay with 21 DM analogues was performed by Hao et al., to identify BMPR or VEGFR biased kinase inhibitors. With no apparent detrimental effect on the zebrafish vasculature, as studied by the formation of intersomitic vessels at 12 h post-infection, DMH1 displayed an improved specificity, although less potency, for ALK2 when compared with LDN-193189 and DM in cell-based and in vitro kinase assays. In contrast, DMH4 lost the dorsalization activity from DM, while increasing its anti-angiogenic effect [127].

In order to identify additional novel small molecules with enhanced affinity for ALK2, an in vitro fluorescence-based thermal stability assay [128] was performed with the kinase domain of ALK2 Q207D and a compound library containing 2000 molecules. As a result, the 2-aminopyridine inhibitor K02288 was discovered, with preferential activity against ALK1 (IC_{50} = 0.8 nM) and ALK2 (IC_{50} = 1.1 nM) using in vitro kinase assays [129] with purified proteins. Of note is that K02288 represents a different scaffold from DM derivatives (Fig. 3B). In order to compare the mode of interaction of K02288 and LDN-193189 with ALK2, the authors co-crystalized the kinase domain of ALK2 in the presence of either compound. Superposition of both co-structures revealed slight differences in the binding mode of the two molecules to ALK2, mainly affecting peripheral residues in the proximity of the ATP binding site, such as K235 (LDN-193189) and E248 (K02288). This may contribute to a higher selectivity of K02288 versus LDN-193189. K02288 was further optimized for BMPR inhibition by Mohedas et al. [130] generating a library of analogues that exhibited different kinome selectivity compared to the original molecule. In particular, the derivative LDN-214117 displayed increased selectivity for BMP type I receptor kinases. Moreover, further structure-activity relationship (SAR) studies were performed aiming to generate new DM derivatives with increased selectivity for BMPR against TGF-β receptors kinases. As such, ML347 (also named ILWY, VU0469381) [131] was identified as a relatively specific inhibitor of ALK1 (IC_{50} = 46 nM) and ALK2 (IC_{50} = 32 nM) in in vitro kinase assays, showing poor selectivity for ALK3 (IC_{50} > 10,000 nM), ALK4, ALK5 and TβR2. Unfortunately, the kinome selectivity of ML347 was not shown. Through a cell-based assay consisting in assessing the activity of constitutively active versions of ALK1, ALK2, ALK3, ALK4 and ALK5, a set of pyrazolo[1,5-*a*]pyrimidine derivatives was tested [132], leading to a novel analogue with improved the affinity for BMP against TGF-β type I receptor kinases. This compound, LDN-212854 (Supplementary Fig. 1B), was synthetized by combining the 5-quinoline moiety of LDN-193719 with the phenyl-piperazine substituent of LDN-193189. Compared with LDN-193189, LDN-212854 showed further improved affinity for ALK1 and ALK2 and slightly improved selectivity when assessed for in vitro activity using a broad panel of kinases, while retaining its potency in cell-based assays. Importantly, LDN-212854 efficiently prevented heterotopic ossification in different animal models [132,133], as well as DIPG [78]. A subsequent study by Newman et al., reported that hydrolysis of DM derivatives (e.g., DMH2) in solution may hamper compounds activity. In order to avoid this effect, JL5 and JL12 were synthesized by replacing

Table 2
Representative scaffolds of small molecule interfering with BMPR.

Compound	Chemical name	Structure	Main targets	Activity assay(s)	Reference(s)
Dorsomorphin, Compound c	6-[4-[2-(1-Piperidinyl)ethoxy]phenyl]-3-(4-pyridinyl)-pyrazolo[1,5-*a*]pyrimidine		IC_{50} values (nM): 19.5 (ALK1), 9.76 (ALK2), 222 (ALK3). ALK6 not shown.	In vitro kinase assay with recombinant kinase proteins (6 μM (^{33}P) γATP).	[118,132,164]
LDN-193189	4-[6-[4-(1-Piperazinyl)phenyl]pyrazolo[1,5-*a*] pyrimidin-3-yl]-quinoline dihydrochloride		IC_{50} values (nM): 1.48 (ALK1), 0.67 (ALK2), 14.3 (ALK3). ALK6 not shown.	In vitro kinase assay with recombinant kinase proteins (6 μM (^{33}P) γATP).	[120,132]
DMH1	4-[6-(4-Propan-2-yloxyphenyl)pyrazolo[1,5-*a*] pyrimidin-3-yl]quinoline		IC_{50} values (nM): 77.9 (ALK1), 12.62 (ALK2), 241 (ALK3). ALK6 not shown.	In vitro kinase assay with recombinant kinase proteins (6 μM (^{33}P) γATP).	[127,132]
K02288	3-[(6-Amino-5-(3,4,5-trimethoxyphenyl)-3-pyridinyl)]phenol		IC_{50} values (nM): 3.65 (ALK1), 1.20 (ALK2), 25.8 (ALK3). ALK6 not shown.	In vitro kinase assay with recombinant kinase proteins (6 μM (^{33}P) γATP).	[129,132]
Compound 1	6-Pyrazole quinazolinone		IC_{50} value for ALK2: 8.2 μM	LanthaScreen Eu Kinase Binding Assay.	[138]
ML347, 1LWY	5-[6-(4-Methoxyphenyl)pyrazolo[1,5-*a*] pyrimidin-3-yl]quinoline		IC_{50} values (nM): 46 (ALK1), 32 (ALK2), 10,800 (ALK3), 9830 (ALK6).	In vitro kinase assay with recombinant kinase proteins (10 μM (^{33}P) γATP).	[131]
VU5350	(7-(4-isopropoxyphenyl)-3-(1*H*-pyrazol-4-yl) imidazo[1,2-*a*]pyridine		K_i values (nM): 1970 (ALK1), 92.3 (ALK3), 895 (ALK6). ALK1 not shown.	In vitro kinase assay with recombinant kinase proteins (10 μM (^{33}P) γATP).	[140]
OD52	4,8-Dimethyl-8,12,15,19,20,23-hexaazatetracyclo[14.5.2.1^{2},^{6}.0^{19},^{22}]tetracosa-1(22),2(24),3,5,16(23),17,20-heptaen-11-one		Kd values (nM): 13 (ALK1), 9.6 (ALK2), 1300 (ALK3), ALK6 not shown.	In vitro thermodynamic affinity (DiscoverX Kd) determination.	[137]
E6201	[(3*S*,4*R*,5*Z*,8*S*,9*S*,11*E*)-14-(ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,19-tetrahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione]		IC_{50} ALK2 ≈ 0.25 μM.	Nanobret target engagement assay in living cells.	[147,148]
Saracatinib	*N*-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine		IC_{50} values (nM): 3.2 (ALK1), 17.4 (ALK2), 30.7 (ALK3), 295 (ALK6). [142]	In vitro kinase assay with recombinant kinase proteins (10 μM (^{33}P) γATP).	[142,165]
BYL719	(2*S*)-N^{1}-[4-methyl-5-[2-(2,2,2-trifluoro-1,1-dimethylethyl)-4-pyridinyl]-2-thiazolyl]-1,2-pyrrolidinedicarboxamide		Nearly complete inhibition at 5 and 10 μM.	BMP2 (2 nM)-induced pSMAD1/5 by western blotting.	[145,166]

an oxygen with a carbon atom in the side chain. Interestingly, whereas JL12 became inactive due to an additional substitution of the R2 position with an imidapyrazole group, JL5's metabolic clearance was reduced by 50% in comparison with DMH2 [134]. The authors conclude that, due to the increased stability of JL5, and it's similar or lower IC_{50} values for ALK2, ALK3, ALK6, ALK5 and TβR2 kinases compared to DM, JL5 was an excellent starting point for development of a bioavailable compound with anti-tumor effects.

Virtual screenings have also led to the identification of molecules that potentially inhibit BMPR kinase activity. Different criteria were considered by Kausar et al. [135] to select 5 lead compounds out of 20,000 available molecules, including compatibility with the ATP pocket, drug-like properties, stability of protein-ligand complexes and binding energy. How these selected inhibitors perform in actual kinase assays remains to be studied.

In addition to DM and K02288 derivatives, many alternative scaffolds have been developed in the past recent years. Macrocyclic inhibitors were synthetized by combining an ATP mimetic scaffold and a functionalized linker [136]. Serendipitously, when macrocyclic inhibitors with high affinity for serine/threonine kinase 2 (RIPK2) were characterized for in vitro selectivity using an array of kinases, the compound OD36 was found to have inhibitory activity towards ALK2 (Fig. 3C). Upon further optimization by modifying the linker region, OD52 was generated, showing enhanced affinity for ALK1 and ALK2. Importantly, these two compounds possessed a more selective kinome profile for BMPRs, when compared with the reference compound LDN-

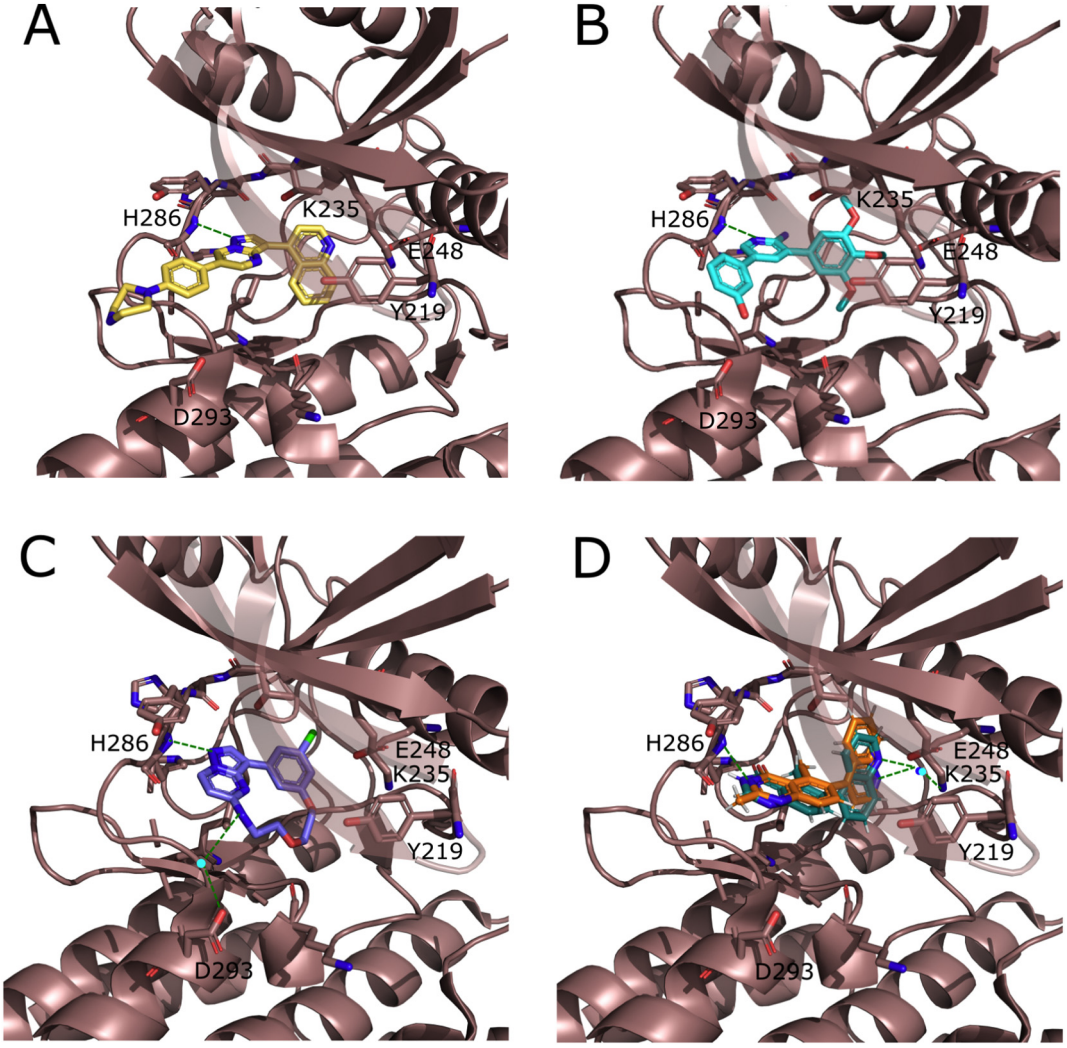


Fig. 3. Comparison of the binding modes of small molecule inhibitors bound to ALK2 as determined crystallographically. In all cases hydrogen bonds are shown with green dotted lines while water molecules are shown with small light blue spheres. A) The binding of LDN-193189 (yellow) to ALK2 (pdb code 3Q4U). B) The binding of K02288 (light blue) to ALK2 (pdb code 3MTF) showing conservation of the key H bond to the hinge region (H286) and occupation of the back of the binding pocket with a tri-methoxy head group. C) Binding of the cyclic compound OD36 (dark blue) to ALK2 (pdb code 5OY6) showing an H bond to the hinge region. D) The reversed binding modes of compounds from Hudson et al. (pdb codes 6GI6 in blue and 6GIP in orange) shown in the ALK2 binding pocket. The overlay shows sufficient flexibility within the pocket for the same water mediated contact toe K235 to be maintained for each compound and that the H bond to the hinge region is retained.

193189. Furthermore, these two compounds effectively prevented ALK2 dependent downstream signaling and osteogenic differentiation in primary cells obtained from patients with FOP [137].

Using an in vitro kinase binding assay, 6-pyrazole quinazolinone was recently identified as a new relatively potent ALK2 inhibitor [138] (IC_{50} = 8.2 μM). Based on this molecule, which shares features with the ALK1 and ALK5 inhibitor PF-03671148 [139], a SAR study directed towards the quinazolinone at position 6, led to several molecules with enhanced potency, and methyl substitutions at the quinazolinone 2, 3 and 5. Interestingly, a series of compounds was found with similar selectivity for ALK2, albeit with distinct in vitro kinome profiles. Crystallography studies led to the identification of two different binding configurations of these novel ALK2 inhibitors, with a 'flipped binding mode' seen which suggests that replacement of the N-1 (the 'usual' hinge binder) with a carbon and additional development at the 2-position of the quinazolinone with a hydrophobic group would be possible (Fig. 3D). In the standard orientation this would clash with the kinase hinge region. When this was tested it resulted in increased potency against ALK2 down to 10 nM. This flipped binding mode's alternative kinase binding profile showed off-target hits limited to the tyrosine kinase-like family, including ALKs 1, 4, 5 and 6, as well as RAF1 and BRAF. Notably, this the first time in which a compound has been developed which is more selective for ALK1 than ALK2.

BMP type I receptors, other than ALK2, have also been targeted for therapeutic gain. VU5350 [140] (7-(4isopropoxyphenyl)-3-(1H-pyrazol-4-yl)imidazo[1,2-*a*]pyridine) was identified as an ALK3-biased inhibitor with beneficial effects in animal models of hepatic damage. In comparison with LDN-193189, which showed Ki values for the BMPRs ALK2, ALK3 and ALK6 below 50 nM, VU5350 was selective for ALK3 (92.3 nM) compared to other BMPRs (ALK2: 1970 nM, ALK6: 895 nM). Importantly, VU5350 also showed a remarkable activity (higher than LDN-193189) against TβR2 (Ki = 85.6 nM), AMPK (Ki = 150 nM) and VEGFR2 (Ki = 14.9 nM). Recently LJ000328 was disclosed as an ALK3 biased inhibitor (IC_{50} = 5.1 nM as determined using in vitro kinase assays), with low affinity for TGFβ type I and type II receptors [141]. This novel compound was tested using in vivo models of iron-refractory iron-deficiency anemia (IRIDA). The authors demonstrated that intraperitoneal injection of LJ000328 (20 mg/kg for up to 7 weeks) partially prevented hepcidin expression and anemia development in *Tmprss6*$^{-/-}$ mice, while being well tolerated with no significant weight

loss.

Identifying and characterizing inhibitory molecules with high selectivity for BMPRs is not only highly interesting to interrogate BMPR signaling, but also to develop safe therapies with minor side effects for BMPR-related diseases. Such highly selective compounds may be combined with alternative drugs thereby achieving synergistic effects. Interestingly, a number of molecules originally developed for other purposes were found to interfere with BMPR signaling. For example, AZD0530 (Saracatinib) initially targeted against Src and Abl [142–144], the phosphoinositol-3-kinase (PI3K) inhibitor BYL719 [145], and the ERB2 antagonist TAK-165 [144], target BMPR kinase activity. The RAR-γ agonist Palovarotene, induces degradation of SMAD1 [146], the downstream effector of BMPRI. E6201 [(3*S*,4*R*,5*Z*,8*S*,9*S*,11*E*)-14-(ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,19-tetrahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione], that had been previously characterized as a mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK)-1 and MEK kinase-1 [147], was found to also inhibit ALK2 [148]. While the binding of E6201 to ALK2 retains the same hinge binding contacts and hydrophobic interactions compared to MEK kinase, it lacks the covalent bond that makes E6201 binding irreversible. The substitution of Cys207 in MEK for A353 in ALK2 results in a reversible mode of inhibition (Supplementary Fig. 1C). This dual activity made E6201 a good candidate drug for DIPG, where E6201 targets both overly active PDGF receptor-induced MEK pathway and mutated ALK2. E6201 prevented the growth of patient-derived tumor cells and improved prognosis in animal disease models. This illustrates how drug repurposing may be a valid alternative approach to identify compounds with potent anti-BMPR activity. Furthermore, such repurposed drugs may bear an added value as in occasions previous studies have reported additional information, including pharmaco-kinetics and -dynamics, potential side-targets, and toxicity.

At this moment, an increasing number of small molecules targeting BMPR activity are being investigated or will shortly enter in clinical trial studies (i.e., BLU782, BCX9250, KER-047, TP-0184, Saracatinib, Palovarotene, BYL719, E6201). Small molecules with enhanced kinase inhibitory selectivity, good drug-like properties, and hopefully enhanced tolerability have been developed. Studies of such compounds have revealed promising safety results; moreover, toxicity may be lowered even more by using combination of drugs at a reduced dose, local delivery and intermittent administration, for example. This kind of pharmacological agents targeting BMPR kinase activity may be complemented by or combined with other tools interfering with BMPR signaling at different levels. For example, recent developments using ECD-based ligand traps have reported promising results in preclinical models of PAH [149], atherosclerosis and vascular calcification [123], cancer [28,150] and heterotopic ossification [133,151] or have advanced into clinical trials, such as Dalantercept (NCT01458392, NCT02024087, NCT01642082, NCT01720173, NCT01727336, NCT00996957) as an ALK1-based ligand trap for different varieties of cancer, Sotatercept (PAH NCT03496207, Cancer NCT01736683) and ACE-011 (Chemotherapy-induced anemia NCT00931606, Osteoporosis NCT00709540) for an ActRIIa-based ligand trap.

5. Conclusions and perspectives

The BMP family consists of more than ten dimeric isoforms that exert their cellular effects by binding to hetero-tetrameric complexes of type I and type II serine/threonine kinase receptors, of which there are four and three, respectively. BMP ligands can be divided in different structurally related subgroups, the BMP-2/-4 group, the BMP-5/-6/-7/-8 group, the BMP-9/-10 group, and the BMP-12/-13/-14 group. Within each subgroup there is high overlap in receptor binding specificity. In addition, there is a considerable promiscuity in receptor binding. Each ligand binds to more than one type I receptor and often more than one type II receptor, and each receptor binds more than one ligand. All these BMPs, acting via BMP receptors, converge on SMAD1, -5 and -8, which upon phosphorylation form transcription factor complexes with SMAD4, as well as activating certain non-SMAD (non-canonical) pathways.

BMPs are expressed in a large variety of tissues and steer the differentiation and function of a broad range of different cell types that are highly dependent on the intensity and duration of the BMP signal. Each step of the BMP pathway is subjected to intricate regulation by extracellular and intracellular cues. The function of BMP receptors is also controlled in multiple ways. For instance, co-receptors promote or suppress ligand presentation, and inhibitory members of the SMAD family (SMAD6 and -7) are induced by BMP stimulation and act in a feedback mechanism to mediate BMPR ubiquitination and promote proteasomal degradation. An important remaining question is how BMPR-induced intracellular specificity via canonical SMAD and non-canonical pathway activation is achieved. Furthermore, the possibility that the type II receptors, known to phosphorylate and activate the type I receptors, have additional roles in signaling, deserves further exploration. Another aspect which should be given attention is the possibility that hetero-tetrameric complexes, consisting of two different type I and/or two different type II receptors, are formed and have specific signaling properties.

Human mutations in BMP receptors have been causally linked to diverse diseases, including muscular-skeletal disorders, cardiovascular diseases and cancer to name a few. Moreover, gain-of-function or loss-of-function of different BMP receptors in animal models has been linked with various pathologies. To treat diseases which involve over-activity of BMP signaling, different kinds of inhibitors are being developed. For each condition, it remains to be determined whether inhibition of ligand or receptor by antibodies or other binders, inhibition of the kinase activities of the respective receptors, or induced degradation of signaling molecules, e.g. by the proteolysis targeting chimeras (PROTAC) method [152], will provide the most efficient treatment.

With regard to low-molecular-weight kinase inhibitors, a significant challenge is to develop inhibitors that selectively inhibit any given receptor kinase(s). In certain diseases, e.g. FOP, it is beneficial that the inhibitor selectively targets only ALK2 to minimize toxic side effects. For some diseases in which there is a pathological involvement of multiple kinases, an inhibitor that selectively targets multiple kinases could be an advantage. Due to the high conservation of the ATP binding pocket among cellular kinases, selectivity may be improved by the development of so-called allosteric inhibitors, which target kinase specific residues not involved in ATP binding but in kinase activation. It should also be noted that much of the non-canonical signaling is not dependent on the kinase activity of the receptors, and therefore will not be impacted by kinase inhibitors. Depending on the disease context, this may be an advantage or a disadvantage.

While in this review we discussed BMPR inhibitors, activators may also have therapeutic benefit, for example for the treatment of PAH, characterized by inactivating mutations in *BMPR2*, or in cases of cancer where activating BMP signaling inhibits epithelial to mesenchymal transition and invasion. The immune suppressant FK506 (Tacrolimus) was identified from an FDA-approved drug library through a BMP transcriptional reporter screen for its ability to induce BMPR signaling and reverse PAH. At very low concentrations FK506 was able to induce SMAD1/5 phosphorylation and restore endothelial function in PAH cells by preventing the interaction of BMPR1 with FKBP12 [153]. Moreover, FK506 was found to improve vascular pathology [154] and restrain bladder cancer progression [155]. Unfortunately, FK506 suppresses the immune response, an effect that is not desirable for the treatment of aforementioned diseases. Of note, a FK506 analogue, termed FKVP, is not as immune suppressive, but still retains its ability to activate BMPR signaling [156]. Additional BMP agonists were identified out of a compound library with over 600,000 compounds using a BMP-dependent transcriptional reporter screen in human cervical carcinoma cells. Because of their ability to induce ventralization in

zebrafish embryos, these compounds were termed ventromorphins [157]. In line with these observations, multiple developing (semi)synthetic compounds are emerging with the ability to induce BMPR activation [158–162]. Nevertheless, one should note that over-activation of BMPR signaling may lead to undesirable side effects, which need to be carefully considered.

The field of BMP receptor research has undergone significant maturation and now we have a clearer picture of BMP receptor signaling mechanisms, in vivo function and role in diseases. The field is rapidly evolving, and the many remaining detailed questions are being answered at a high speed. With the recent development of the translational aspects, it is anticipated that modulators of BMP receptor signaling will soon reach the clinic for the treatment of musculoskeletal and cardiovascular diseases.

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Declaration of competing interest

The authors declare no competing interests.

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