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Emerging roles of the bone morphogenetic protein pathway in cancer: potential therapeutic target for kinase inhibition

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Abstract

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor- β (TGF- β) family signalling pathway. Similar to TGF- β , the complex roles of BMPs in development and disease are demonstrated by their dichotomous roles in various cancers and cancer stages. Although early studies implicated BMP signalling in tumour suppressive phenotypes, the results of more recent experiments recognize BMPs as potent tumour promoters. Many of these complexities are becoming illuminated by understanding the role of BMPs in their contextual role in unique cell types of cancer and the impact of their surrounding tumour microenvironment. Here we review the emerging roles of BMP signalling in cancer, with a focus on the molecular underpinnings of BMP signalling in individual cancers as a valid therapeutic target for cancer prevention and treatment.

Keywords

bone morphogenetic protein (BMP) signalling; cancer; small molecule BMP inhibitors

BMP signalling pathway: a primer

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor- β (TGF- β) family. First described by their ability to induce ectopic bone formation [1,2], BMPs are now recognized as multifunctional cytokines essential for defining the vertebral embryonic axis during development and regulating key biological processes, such as endochondral bone formation, organogenesis and angiogenesis. To date, there are over 20 known BMP ligands (Figure 1). BMPs are synthesized as large inactive precursors (400–500 amino acids) comprise an N-terminal signal peptide, prodomain that ensures proper folding

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C.C.H. holds patents and patent applications on composition of matter and methods of use of small molecule BMP inhibitors, and has licensed the technology to La Jolla Pharmaceutical Co. for clinical development.

and a mature C-terminal peptide [3,4]. Sequential cleavage of two sites within the prodomain (R-X-K-R and R-X-X-R respectively) by furin and procollagen C-proteinase (also known as BMP1) yields the mature BMP monomer (50–100 amino acids) [5]. The mature BMP monomer has seven cysteines; six form intramolecular disulfide bonds, whereas the seventh dimerizes with another monomer to generate the biologically active BMP ligand, which exists as homo- and heterodimers [6,7].

All members of the TGF- β superfamily, including BMPs, bind to and recruit two type I and two type II serine/threonine receptor kinases to form a transmembrane receptor complex [5,7,8] (Figures 1 and 2). Upon formation of this complex, the constitutively active type II receptor phosphorylates the type I receptor, activating its kinase domain [9] (Figure 2). Activated type I receptors then signal via the SMAD family of downstream mediators [9]. SMADs are divided into three distinct classes: (1) receptor regulated SMADs (R-SMADs); (2) common-mediator SMAD (Co-SMAD) and (3) inhibitory SMADs (I-SMADs). The R-SMADs (SMAD1, -2, -3, -5 and -8) are directly phosphorylated and activated by type I receptors. R-SMADs, in turn, active SMAD4, the sole Co-SMAD, and, together, translocate to the nucleus to activate downstream BMP target genes. The I-SMADs (SMAD6 and -7), in contrast, antagonize TGF- β and BMP signalling [9]. Independent of the canonical SMAD-mediated signalling pathway, BMPs can also participate in a non-canonical pathway that leads to activation of the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) signalling cascade [10].

As shown in Figure 1, TGF- β signalling is very complex, involving the engagement of multiple type I and type II receptors and R-SMADs [5]. In general, BMP ligands engage three type II receptors (Table 1): activin receptor, type IIA, (ActR-IIA); activin receptor, type IIB (ActR-IIB); and BMP receptor, type II (BMPRII) [5,8]. In addition, BMPs engage four type I receptors (Table 1): activin receptor like kinase-1 (ALK1), also known as activin A receptor type II-like 1; activin receptor like kinase-2 (ALK2), also known as activin A receptor, type I; activin receptor like kinase-3 (ALK3), also known as BMP receptor, type 1A; and activin receptor like kinase-6 (ALK6), also known as BMP receptor, type 1B [5]. BMP type I receptor activation leads to phosphorylation of SMAD1/5/8, which translocates to the nucleus to complex with SMAD4 and activate target genes, such as the inhibitors of DNA binding/differentiation (Id) family [5] (Figure 2). The Id proteins, comprise Id1, Id2, Id3 and Id4, are key regulators of cell proliferation and are implicated in tumorigenesis through their pro-apoptotic and oncogenic properties.

BMP signalling is regulated at multiple levels, including tissue distribution of ligands and receptors, assembly of distinct transmembrane receptor complexes by numerous ligand homo- and heterodimer combinations [5], and extracellular protein antagonists that directly bind to BMP ligands with distinct affinities. The extracellular BMP antagonists are classified into three subfamilies: 1) DAN family, which includes Gremlin, DAND5 (COCO) and Sclerostin (SOST1); 2) Twisted gastrulation (TSG) and 3) Chordin and Noggin [11]. BMP signalling is also regulated by intracellular factors such as the I-SMADs, SMAD ubiquitination regulator factors (Smurfs), and serine/threonine phosphatases, such as PP1A [12]. The sheer complexity of BMP-TGF- β signalling, involving multiple extracellular antagonists, multiple forms of ligand homo-heterodimers, complex ligand–receptor

interactions, and overlap between the canonical BMP and TGF- β signalling pathways, poses a significant challenge towards identifying appropriate targets for therapeutic development.

Role of BMP signalling in cancer pathogenesis

Although a relationship between BMPs and cancer has been noted for over 20 years, the precise roles of BMP signalling in cancer development and progression are just beginning to be elucidated. Depending on the BMP ligand and cancer type, BMPs can either promote or inhibit tumorigenesis. This review serves to further elucidate the role of the BMP pathway and shed light on the recent development and use of small molecule BMP inhibitors for potential cancer therapy. Here, we present an updated review of BMP function in specific cancers (Table 2) and discuss the potential of the BMP pathway as a therapeutic target.

Colorectal cancer

BMP ligands and their receptors are found in mesenchymal and epithelial cells within the colon and small intestine and are actively involved in apoptosis of mature colonic epithelial cells and maturation of small intestinal secretory cells [13]. Consistent with the important role of BMP signalling in gastrointestinal homeostasis, specific gene mutations encoding components of the BMP pathway are associated with gastrointestinal cancer [14,15].

Juvenile Polyposis Syndrome (JPS, OMIM#174900) is an autosomal dominant syndrome that predisposes individuals to upper gastrointestinal polyps and a significantly increased lifetime risk of developing colorectal cancer. Germline mutations in *BMPRI1A* (encoding ALK3) and *SMAD4* genes account for nearly half of all JPS cases [16–18]. In histological tissue analysis of polyps from patients with JPS and a *SMAD4* germline mutation, epithelial SMAD4 protein expression was absent from polyps, supporting an important role of BMP signalling in the development of epithelial hamartomas [19]. In *in vivo* studies of transgenic mice, forced expression of the BMP antagonist Noggin resulted in the formation of intestinal hamartomas that were morphologically similar to those found in human JPS [20]. Similarly, *BMPRI1A* mutations in mice also induced intestinal hamartomatous polyps [21]. Taken together, both human and murine studies suggest that the germline loss of ALK3 and SMAD4 increases the risk of spontaneous gastrointestinal cancer.

The role of BMPs in sporadic colorectal cancer (CRC), however, is less well defined. In *in vitro* models, BMP2, BMP3 and BMP7 were tumour suppressive, and inactivation of the *BMP3* gene through promoter methylation was associated with microsatellite instability and *BRAF* oncogene mutation [22,23,24]. In addition, several studies demonstrated a widespread loss of BMP function in epithelial cells of CRC. For instance, using phosphorylated-SMAD1, -5, -8 (p-SMAD 1/5/8) as a marker of BMP pathway activation, Kodach et al. [25] demonstrated that BMP signalling was inactivated in 70% of CRC specimens. From their study, Kodach et al. [25] hypothesized two pathways by which BMP loss-of-function leads to CRC: (1) loss of epithelial SMAD4 epithelial expression and (2) loss of epithelial BMPR2 expression. In CRC cell lines, loss of BMPR2 expression correlated with microsatellite instability and disruption of the 3'-untranslated region (3'UTR) of the *BMPR2* gene [26]. At the same time, however, BMPR2 activation also correlated with more advanced tumour grade [26]. In addition, increased expression levels of

BMP4 and BMP7 were associated with tumour progression as well as poorer clinical prognosis [27,28]. Interestingly, in contrast with the association of germline *BMPR1A* mutation with polyp formation, *ALK3 (BMPR1A)* loss-of-function did not lead to CRC [23]. In summary, loss of BMP signalling appears to be associated with early stages of tumorigenesis at the transition from adenoma to carcinoma, whereas BMP signal activation is associated with high-grade advanced tumours.

Recent finding that loss of SMAD4 in CRC cells switches BMP signalling from tumour suppressive to metastasis promoting may reconcile of some of the divergent roles of BMP signalling in CRC [14]. In CRC patients, loss of SMAD4 in the presence of normal BMP expression is associated with reduced survival times. In CRC cells lacking SMAD4, activation of BMP signalling promotes metastatic behaviour via activation of Rho signalling via ROCK and LIM domain kinase (LIMK). These results indicate the role of SMAD-independent non-canonical BMP pathway in CRC pathogenesis, and suggest that pharmaceuticals that block this pathway, such as ROCK inhibitors, might be efficacious for CRC [14].

Acute megakaryoblastic leukaemia (AMKL)

Acute megakaryoblastic leukaemia (AMKL; M7) is a clinically heterogeneous disease that represents 5–10% of all paediatric acute myeloid leukaemia (AML) and 1–2% of adult AML [29–31]. In children, AMKL is classified into two major subgroups: leukaemia in patients with Down syndrome (DS-AMKL) and leukaemia in patients without Down syndrome (non-DS-AMKL). DS-AMKL carries a very favourable prognosis with an event-free survival rate close to 80% [29,32]. Patients with non-DS-AMKL, however, fare considerably worse and, until recently, little was known about the genetic aberrations that underlie non-DS-AMKL.

Using transcriptome sequencing, Gruber et al. [33] found that 27% of patients with non-DS-AMKL had a cryptic inversion on chromosome 16, *inv(16)(p13.3q24.3)* that resulted in the fusion of *CBFA2T3* to *GLIS2*. In addition, patients with *CBFA2T3-GLIS2* had significantly reduced survival compared with patients without the chimeric transcript (28% compared with 42% 5-year survival) [33]. *CBFA2T3*, a member of the ETO family of transcriptional corepressors, is normally expressed in the hematopoietic system and is involved in maintaining hematopoietic stem cell quiescence [34]. Unlike *CBFA2T3*, *GLIS2* is not normally found in the hematopoietic system; rather, *GLIS2* is a member of the Kruppel-like zinc finger transcription factor family that is implicated in the regulation of normal renal development and function [35,36]. Interestingly, gene expression profiling showed that non-DS-AMKL patients with the *CBFA2T3-GLIS2* fusion had marked overexpression of *BMP2* and *BMP4* [33].

Further functional studies confirmed activation of the BMP signalling pathway via the canonical SMAD-dependent pathway through *in vitro* expression of downstream BMP targets and *in vivo* BMP gain-of-function phenotypes [33]. Transfection of *CBFA2T3-GLIS2* in murine hematopoietic cells increased expression of *Id1* and promoted cell self-renewal, both of which were inhibited by the small molecule BMP inhibitor dorsomorphin (DM) [33] (Figure 3). AMKL cell lines that expressed *CBFA2T3-GLIS2* also had reduced cell viability and proliferation in the presence of DM compared with AMKL cell lines that

lacked the fusion transcript [33]. Finally, expression of CBFA2T3-GLIS2 in *Drosophila* led to increased expression of endogenous *dpp*, the fly homologue of BMP4, resulting in shortened, blistered wings and ectopic wing veination, phenotypes consistent with *dpp* gain-of-function [33,37]. Because BMPs are known to be involved in hematopoietic differentiation, and increased BMP signalling has been shown to induce CD34 + progenitor differentiation into megakaryocytes [38], the critical importance of BMP signalling in AMKL pathogenesis is not surprising.

Chronic myelogenous leukaemia (CML) and other leukaemias

CML is driven by the oncogenic tyrosine kinase fusion protein BCR (breakpoint cluster region)-Abl (Abelson murine leukaemia viral oncogene homologue 1) [39]. Treatment of CML with small molecule tyrosine kinase inhibitors (TKIs) that inhibit BCR-Abl, such as Imatinib, has been transformative in controlling CML for the majority of patients [40]. However, currently available TKIs do not eliminate quiescent leukemic stem cell populations [41]. Consequently, long-term suppressive TKI treatment is required to sustain remission, and tumour recurrence occurs as leukemic cells acquire resistance to BCR-Abl inhibitors [42]. Therefore, recent attention has focused on discovery of new therapeutic approaches to target additional signalling pathways in the leukemic stem cell population.

The BMP pathway plays an important role in hematopoietic stem cell (HSC) renewal within the bone marrow (BM) niche, and deregulated BMP signalling has been observed in various hematologic malignancies, including CML [42]. Although exact details of mechanisms are yet to be fully elucidated, recent studies indicate that alterations in the BMP pathway act in a paracrine fashion to enhance proliferation and survival of primitive leukemic stem cells and more mature myeloid progenitors [42,43]. In addition, BMP signalling appears to have oncogenic properties for promyelocytic leukaemia and acute myelogenous leukaemia, presumably via similar effects on leukemic stem cells [44,45]. Taken together, emerging evidence suggest that BMP inhibition may represent a novel approach for leukaemia treatment by targeting leukemic stem cells, perhaps in conjunction with existing targeted therapies.

Diffuse intrinsic pontine glioma and other high-grade gliomas

Diffuse intrinsic pontine glioma (DIPG) is the leading cause of death in children with brain tumours and carries a poor prognosis with a median survival time of less than 1 year [46]. Surgical resection is not feasible due to the challenging anatomical position of the tumour and, to date, chemotherapy, radiation and targeted agents have not been effective [47], prompting efforts to better understand the molecular genetics underlying DIPG.

BMPs are actively involved in neurogenesis [48] and activation of the BMP signalling pathway has recently been implicated in the pathogenesis of DIPG. Four concurrent, independent studies identified recurrent somatic mutations in the *ACVR1* gene, which encodes ALK2, in 46 of 195 DIPG samples [49–52]. These gain-of-function mutations are due to seven different amino acid substitution: R206H, Q207E, R258G, G328V, G328W, G328E and G356D. Interestingly, with the exception of R258G and G328V, the *ACVR1* mutations in DIPG are identical with the germline mutations found in fibrodysplasia

ossificans progressiva (FOP), an autosomal dominant disorder characterized by heterotopic ossification of soft tissues [53]. Since the FOP *ACVR1* mutations promote BMP signalling through increased phosphorylation of R-SMADs and increased expression of downstream transcriptional targets [54], DIPG tumour growth and resistance to therapy could be driven by aberrant BMP signalling in a cell autonomous fashion.

Indeed, *in vitro* and *in vivo* studies demonstrated activation of BMP signalling in *ACVR1* mutant DIPG through increased levels of phosphorylated SMADs and Id1 and Id2 expression. Human glioma specimens with *ACVR1* mutations expressed higher levels of p-SMAD1/5 compared with wild type controls [49,50]. Transfection of mutant versions of *ACVR1* in mouse astrocytes also resulted in increased levels of p-SMAD1/5 [49]. Furthermore, treatment of the same mutant *ACVR1* astrocytes with the small molecule BMP inhibitor LDN-193189 (Figure 3) inhibited SMAD phosphorylation [49]. *ACVR1* mutant human astrocytes also expressed increased levels of Id1 and Id2, confirming downstream activation of the BMP pathway, and increased proliferation of cultured brainstem progenitor cells [50]. In *in vivo* zebrafish experiments, expression of mutant *ACVR1* disrupted embryologic dorsoventral patterning and induced ventralization and loss of head and dorsal structures. Moreover, addition of LDN-193189 partially rescued the ventralization phenotype, providing further evidence that the DIPG-associated mutations activate ALK2 [49].

Interestingly, although these studies demonstrated that *ACVR1* mutations drive DIPG tumorigenesis, these same somatic mutations that are seen in FOP patients do not lead to cancer. To date, there are no reported cases of DIPG in FOP patients. Furthermore, when expressed in mouse astrocytes, mutations in *ACVR1* associated with DIPG do not induce tumorigenesis [49], suggesting that whereas activation of BMP signalling is necessary for development of the oncogenic phenotype, additional molecular aberrancies are also required.

Recently, we demonstrated that BMP signalling is active in the majority of high-grade glioma (HGG) cells obtained from adult patients [55]. Moreover, consistent with a role of BMP signalling in human HGG, deletion of *BMPRI1A* (ALK3) in oncogenic mouse astrocytes resulted in decreased proliferation, invasion and migration. In addition, in a mouse model of HGG involving orthotopic transplantation of transformed astrocytes, the genetic knockout of *BMPRI1A* (ALK3) showed a dramatic increase in survival. Finally, much of the *in vitro* effects of *BMPRI1A* ablation were recapitulated using a small molecule BMP inhibitor DMH1. Taken together, these results suggest that BMP signalling may play an important role in the pathogenesis of HGG and that pharmacological agents such as DMH1 may be efficacious for treatment of a subset of adult HGG driven by BMP signalling.

Lung cancer

Lung cancer is the leading cause of cancer-related deaths in the United States. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Although BMP2 is essential for lung development, normal mature lung cells do not produce BMP2 [56]. BMP2 is aberrantly overexpressed in 98% of NSCLC, and increased levels of p-SMAD 1/5 and Id1 are reported in NSCLC biopsies [57]. As such, the role of BMP signalling in NSCLC has been the subject of much research.

Of the >20 BMP ligands, BMP2 has an established prooncogenic role in the development of NSCLC [58]. Patients with advanced NSCLC have significantly elevated BMP2 levels compared with that of health controls as well as higher metastatic burden and decreased survival [58,59]. Patients with lymph node metastasis and more advanced tumour stages also have higher levels of *BMP2* gene expression [60]. In genomic studies, single nucleotide polymorphisms (SNPs) in the *BMP2* gene associated with unfavourable response to therapy and decreased survival. For example, homozygous carriers of the SNP rs235756 had a significantly shorter mean survival time compared with that of either wild type or heterozygous carriers [61]. In addition, *in vitro* studies demonstrated that BMP2 can stimulate cell growth and invasion in A549 and H7240 lung cancer cell lines in a dose-dependent manner, an effect that can be completely inhibited by the BMP2 antagonist, Noggin [62]. Furthermore, BMP2 increased tumour growth [62] and enhanced tumour angiogenesis in murine lung cancer models [63].

Small molecule BMP inhibitors have been utilized to further explore the role of BMP signalling in lung cancer. In the H1299 and A549 lung cancer cell lines, which express endogenous levels of BMP, small molecule BMP antagonists (Figure 3) significantly decreased phosphorylation of SMAD1/5/8 and expression of *Id1-3* [64]. Importantly, inhibition of BMP type I receptors by small molecule inhibitors resulted in significant growth inhibition and cell death in H1229 and A549 cells [64]. Thus, the use of selective small molecule BMP inhibitors demonstrated the therapeutic potential of blocking the canonical SMAD-dependent pathway in lung cancer.

Pancreatic cancer

Pancreatic cancer is an aggressive tumour with a high mortality rate and limited treatment options, due in part to the late diagnosis for non-specific symptoms and poor response to chemotherapy. Despite current therapies for pancreatic cancer, the cure rate is less than 5% [65]. Given the importance of BMP in the pathogenesis of other solid tumours, there has been growing interest in studying BMP signalling in pancreatic cancer.

BMPs have a complex role in pancreatic cancer. In primary pancreatic cancer specimens, BMP2, BMP4 and BMP7 are elevated, and BMP treatment increased invasiveness of Panc-1 pancreatic cancer cells via SMAD1-mediated activation of matrix metalloproteinase (MMP)-2, the latter of which promotes tumour invasion by degradation of the extracellular matrix [66]. Of note, in the BMP pathway, SMAD4 is a key mediator of BMP target gene transcription [15] (Figure 2). Mutations in the *SMAD4* (also known as *depleted in pancreatic cancer locus 4; DPC4*) gene occurs in >50% of pancreatic cancers, suggesting a tumour suppressor role for SMAD4 [67–69]. Inactivation of SMAD4 is also associated with poor clinical prognosis, and restoration of SMAD4 in SMAD4-depleted cancer cell lines reduced cell growth [69–71]. In SMAD4-positive pancreatic ductal adenocarcinoma (PDAC), reduction in *BMPRIA* (ALK3) expression increased cell viability, Matrigel invasion, activation of angiogenesis markers and matrix modification [72]. Treatment with BMP2 decreased the viability of SMAD4-positive PDAC cells, but, paradoxically, increased the viability of SMAD4-negative cells. Conversely, treatment with LDN-193189 increased viability of SMAD4-positive cells but decreased viability of SMAD4-negative cells.

Together, these results suggest that BMPs have divergent roles in pancreatic cancer pathogenesis depending on the presence or absence of SMAD4: when SMAD4 is present, BMP acts as a tumour suppressor; whereas in its absence, BMP becomes a tumour promoter [72]. Thus, small molecule BMP inhibitors may be selectively therapeutic for SMAD4-negative pancreatic cancer.

Ovarian cancer

Ovarian cancer is the most lethal gynaecologic malignancy. Epithelial ovarian cancer (EOC) accounts for the majority of ovarian cancer and has the highest mortality rate for ovarian cancer [73]. Treatment for EOC includes surgical debulking and/or chemotherapy. Although the vast majority of patients are responsive to chemotherapy, up to 75% of patients will relapse within the first 18 months [74]. Unfortunately, recurrent disease is not curable.

A number of studies indicate that BMP signalling has protumorigenic roles in ovarian cancer. In ovarian cancer cell lines, autocrine stimulation by BMP4 up-regulated Id1 and Id3 expression [75,76]. In addition, human ovarian cancer cells had a nearly four-fold increase in Id3 expression compared with normal primary human ovarian surface epithelial cells [75]. Consistent with the tumour promoting role of BMP, BMP2 expression in high-grade EOC was associated with a poorer prognosis and a significantly shorter survival period [77]. Studies on mesenchymal stem cells (MSCs), multipotent progenitor cells that display tropism towards tumours, [78] have also shed further light into the role of BMP in ovarian cancer. In patients with primary ovarian cancer, >90% of tumour samples expressed carcinoma-associated MSCs (CA-MSCs). In addition, MSCs isolated from human tumour samples promoted tumorigenesis in immune deficient mice [79]. Interestingly, CA-MSCs had increased BMP2 expression compared with control MSCs [79]. Ovarian tumours grown with CA-MSCs also had increased levels of p-SMAD1/5 compared with tumours grown with adipose-MSCs. Similarly, addition of BMP2 to tumours cells increased levels of p-SMAD1/5, an effect that was attenuated by the BMP antagonist Noggin [79]. Taken together, these results suggest that disrupting both autocrine and paracrine BMP signalling may be a therapeutic approach for EOC.

More recently, the functional activation of the BMP signalling pathway was examined in patients with serous ovarian cancer [80]. Gene expression profiling demonstrated that up-regulation of *SMAD5* was associated with poorer prognosis. In addition, *SMAD5*, *BMPRI1A*, *BMPRI1B* and *BMPRI2* expression all positively correlated with *MKI67* (marker of proliferation Ki-67). Furthermore, immunohistochemistry studies demonstrated that nuclear p-SMAD5 level inversely correlated with clinical prognosis: overall survival of patients with high nuclear p-SMAD5 levels was significantly lower than that of patients with low nuclear p-SMAD5. In a mouse model of serous ovarian cancer, administration of recombinant BMP2 also promoted significant tumour growth compared with that of control mice, an effect that was inhibited by DM. Nuclear p-SMAD5 level was also highest in tumour tissue from mice treated with recombinant BMP2 and lowest in tumour tissue from mice treated with DM. A significant positive correlation between p-SMAD5 levels and expression of Ki67 was also observed. [80] These studies highlight the potential therapeutic use of small molecule BMP inhibitors in ovarian cancers.

Osteosarcoma

Osteosarcomas are the most common primary bone malignancies in children and young adults and accounts for more than 50% of bone cancers in individuals under the age of 20 years [81]. Because of their importance in bone and cartilage development, BMPs have been studied in the pathogenesis of osteosarcoma.

BMP ligands and receptors have been detected in the majority of osteosarcoma cell lines. Moreover, human osteosarcoma samples demonstrated activated BMP signalling as measured by phosphorylated SMAD1 levels [82]. Interestingly, human osteosarcoma specimens expressed different levels of BMP expression depending on the subtype of osteosarcoma. For example, high-grade osteosarcoma with malignant fibrous histiosarcoma (MFH)-type pattern had increased BMP2 and BMP4 expression [83], whereas chondroblastic osteosarcoma had increased BMP6 expression [84].

Studies found that osteosarcomas with increased BMP signalling were more chemo-resistant and had a higher likelihood of metastasis, resulting in a reduced 5-year survival rate [85]. For instance, increased expression of BMP2 in osteosarcoma tumours was associated with a higher tendency for tumour metastasis [86], and patients with increased co-expression of BMP2, BMP4 and BMP6 had a poorer prognosis and significantly reduced 5-year survival rate [87]. In genomic studies, individuals homozygous for the SNPs rs3178250 and rs1005464 in the *BMP2* gene were at increased risk for osteosarcoma [88]. In addition to the human observational studies, functional studies demonstrated the role of BMP signalling in osteosarcoma disease progression. In human osteosarcoma cell lines, BMP2 or BMP9 overexpression promoted tumour cell growth, suggesting that BMPs are pro-mitogenic in osteosarcomas [89]. Moreover, BMP2 treatment promoted osteosarcoma cell migration and invasion whereas the BMP antagonist Noggin abrogated the stimulatory effects of BMP2. Taken together, these studies suggest that BMP signalling plays an important role in osteosarcoma pathogenesis and disease progression [90].

Prostate cancer

Prostate cancer is the most common noncutaneous malignancy in men in the United States. Bones of the axial skeleton are the predominant site of prostate cancer metastasis and are primarily osteoblastic lesions [91]. Complications from bone metastasis include pathologic fractures, spinal cord compression and bone pain. Unfortunately, bony metastasis portends advance disease and treatment is often only symptomatic. Because BMPs are potent osteoinductive factors and bone metastasis is predominantly osteoblastic, expression of BMP signalling in prostate cancer has been the subject of much investigation.

mRNA levels of BMP1–6 have been measured in prostatic tissue from patients with benign prostatic hyperplasia (BPH), non-metastatic prostatic adenocarcinoma and metastatic prostatic adenocarcinoma [92]. BMP6 expression was present in over half of prostate tissue specimens from patients with metastatic prostatic carcinoma. However, BMP6 expression was notably absent from patients with non-metastatic prostate carcinoma or BPH [92]. Subsequent studies demonstrated up-regulation of BMP6 mRNA and protein expression in bone metastasis as well as high-grade tumours [93,94]. In addition, higher-grade prostate

tumours, characterized by a Gleason score of 6 or more, exhibited more intense BMP6 immunostaining compared with lower-grade prostate tumours with Gleason scores of 4 or less [95]. BMP6-positive prostate cancer samples also had increased epithelial staining for Id1, indicating activation of BMP signalling [96]. Interestingly, although BMP6 did not alter prostate tumour cell growth *in vitro*, BMP6 did increase tumour invasiveness [97]. In addition, bone metastasis in prostate cancer appears to mediate cross-talk between the Wnt and BMP signalling. Wnt signalling up-regulated both BMP4 and BMP6 expression in prostate cancer cells and promoted pro-osteoblast activity in both BMP-dependent and BMP-independent mechanisms [97].

Unlike that of BMP6, the expression levels of BMP2, BMP4 and BMP7 in the same study were low to absent from primary prostate cancers [92]. In addition, expression of BMP receptors was decreased in more aggressive prostate cancers [98,99]. However, subsequent *in vitro* studies demonstrated that individual BMP or BMPR expression did not always correlate with functional activity. For example, although BMP2 expression was down-regulated in advanced prostate cancer [100], addition of BMP2 promoted cell invasiveness of C4-2B, LnCaP and PC-3 prostate cell lines [97,101]. In addition, although BMP7 expression was low to absent from primary tumours, BMP7 expression was increased in bone metastatic prostate cancer and overexpression of Noggin inhibited growth of BMP7 positive osteolytic cancer lesions [101]. Moreover, BMP7 enhanced PC-3 tumour cell motility and growth *in vitro* [102]. Although these studies highlight the complexity of BMP signalling in prostate cancer, they also identify the need for further investigation to better elucidate the exact role of BMPs in prostate cancer.

Breast cancer

Breast cancer is the most commonly diagnosed cancer in the United States and is a leading cause of cancer death in women. The propensity of breast cancer to metastasize to bone along with multiple studies demonstrating aberrant BMP expression in breast cancer have prompted further study into the potential role of BMP signalling in the pathogenesis of breast cancer.

Of the BMPs, BMP2 is most studied in breast cancer. In breast cancer cell lines and primary tumours, BMP2 expression levels are consistently low, similar to levels found in normal mammary tissue. Functional studies, however, suggest a dual role for BMP2 in breast cancer: suppressing cancer cell proliferation while also promoting cancer cell invasion and migration.

The antiproliferative effect of BMP2 on breast cancer is associated with up-regulation of SMAD1/4, p21, and the tumour suppressor PTEN (phosphatase and tensin homologue). In MCF-7 human breast cancer cells, BMP2 inhibited oestradiol-induced proliferation of tumour cells through up-regulation of p21, a cyclin-dependent kinase inhibitor, and led to SMAD1/4-mediated arrest of cells in the G1 phase of the cell cycle [103]. BMP2 treatment of MCF-7 cells also increased, in a dose-dependent manner, protein levels of PTEN by inhibiting its degradation [104]. Furthermore, at very high concentrations of up to 1 $\mu\text{g/ml}$, BMP2 also directly inhibited cellular proliferation of human breast tumour cells [105].

At the same time, BMP2 also promotes the motility and invasiveness of breast cancer cells. Brief exposure of MCF-7 cells to BMP2 enhanced tumour cell migration and MCF-7 cells transfected with BMP2 had increased expression of *BCSG1*, a breast cancer-specific gene. In addition, in a mouse xenograft model, BMP2 overexpressing MCF-7 breast cancer cells formed tumours that had increased neovascularization as well as increased Id1 expression and p38 MAPK activation [106,107]. BMP2 also stimulated tumour migration and invasion by inducing expression of an extracellular matrix glycoprotein, tenascin-W, in the tumour stroma [108]. Higher expression of tenascin-W in the stroma around breast cancer lesions portended increased likelihood of metastasis and more aggressive tumour behaviour [108]. BMP2 also induced microcalcification, an important diagnostic marker on mammograms for breast cancer patients, in a dose-dependent manner in a rat xenograft model of breast cancer [109,110].

High BMP4 expression is frequently found in breast cancer cell lines and primary breast tumours [111]. Functional studies indicate that BMP4, like BMP2, has a dual role in breast cancer. For example, in MDA-231 breast cancer cells, BMP4 reduced cell migration and invasion by inhibiting matrix metalloproteinase (MMP) 9 expression [112], and, in a larger panel of breast cancer cell lines and primary breast tumours, BMP4 expression correlated with cell cycle arrest and lower tumour grade [113]. By contrast, BMP4 was found to promote tumour migration and invasion [113], although only in certain cell-lines. BMP4 also enhanced tumour cell invasion by stimulating stromal fibroblasts, an effect inhibited by the BMP small molecule inhibitor DMH1 [114]. Taken together, these studies demonstrate the context dependence of the role of BMP ligands in breast cancer and the challenges in selectively targeting individual BMPs.

In contrast with the dual roles of BMP2 and BMP4 in breast cancer, the receptors ALK3 (*BMPR1A*), ALK6 (*BMPR1B*) and BMPR2 appear to promote cancer progression. In breast cancer patients, elevated ALK3 and BMPR2 expression was associated with a reduced relapse-free survival time [115]. In addition, in a tissue microarray analysis, *BMPR1B* overexpression and SMAD1 phosphorylation were associated with higher tumour grade, more aggressive cancer progression and poorer prognosis [116]. Consistent with the oncogenic role of BMPR1A, in a mouse genetic model expressing MMTV.PyMT oncogene, deletion of BMPR1A impaired mammary tumour formation and metastasis [117]. Finally, treatment with DMH1 reduced lung metastasis in MMTV.PyVMT expressing mice, and the tumours were less proliferative and more apoptotic. In addition, DMH1 treatment altered fibroblasts, lymphatic vessels and macrophages within tumour microenvironment to be less tumour promoting [115]. These results raise the exciting possibility that inhibiting BMP signalling may successfully target both the tumour and the tumour microenvironment to reduce tumour burden and metastasis. Finally, DMH1's effects on tumour infiltration by immune cells suggest that BMP inhibition could enhance the effectiveness of cancer immunotherapies [115].

Like prostate cancer, breast cancer has a tropism to metastasize to the bone. BMP7, which is highly expressed in primary breast cancers, is associated with accelerated bony metastasis in patients with BMP7 positive tumours [118]. Furthermore, the BMP-SMAD transcriptional pathway is activated in bone metastasis from breast cancer. In bone metastasis samples from

breast cancer patients and in a mouse xenograft model, high levels of p-SMAD1/5/8 were observed in comparison with primary tumour cells or lymph node metastasis, suggesting that BMPs specifically mediate metastasis of breast cancer cells to bone [119]. In addition, functional studies demonstrated that expression of dominant negative ALK3 in MDA-231-D prostate cancer cells inhibited phosphorylation of SMAD1/5/8 as well as transcription of Id1. Finally, in a mouse xenograft model, expression of the dominant negative ALK3 significantly reduced the size of bony metastasis and prolonged mean survival time [119].

Melanoma

Melanoma is the most commonly fatal cutaneous cancer and is a major public health burden, increasing at a rate unrivalled by any other cancer [120]. Despite extensive research and the use of chemo- and radiotherapy, the prognosis for metastatic melanoma remains poor.

Melanocytes are derived from neural crest cells and BMP2 and BMP4 are known to promote neural crest cell migration [121]. Compared with that of normal melanocytes, increased mRNA expression of BMP2, BMP6, BMP7 and BMP8 has been observed in 14 different melanoma cell lines. BMP7 expression, in particular, correlated with more aggressive melanoma cell lines [122]. In addition, primary and metastatic melanoma tissue specimens showed increased expression of BMP4 and BMP7 [123]. Functional studies demonstrated that BMP2 and BMP4 play an important role in melanoma cell invasion. In chick embryos transplanted with B16-F1 melanoma cells, BMP2 treatment facilitated cell migration, which was abrogated by treatment with Noggin [124]. In addition, specific inhibition of BMP4 through antisense RNA resulted in significant inhibition (70%) of cellular invasion [123]. In melanoma cell lines, BMP4 also acted as a chemoattractant and promoted angiogenesis, features that were in part mediated by up-regulation of Id-1 [125]. Additional studies showed that BMP2 and BMP4 mediated tumour cell invasion and migration by increasing fibroblast production of matrix metalloproteinases (MMPs) 1, 2, 3 and 13 [126]. Further studies are warranted, but these preliminary studies suggest that the use of small molecule BMP inhibitors may offer a novel therapeutic strategy for this highly aggressive and fatal disease.

Head and neck cancer

Head and neck squamous cell carcinoma (HNSCC) is one of the leading causes of cancer-related deaths. Advanced HNSCC is characterized by aggressive destruction of surrounding tissues and, despite multimodal treatment, prognosis remains poor [127].

BMP2 expression has been detected in tongue and gingival squamous cell carcinoma cell lines as well as benign and malignant salivary gland tumours [128,129]. Compared with normal mucosa, human oral squamous cell carcinoma (SCC) tumours overexpress *BMP2*, *BMP4*, *BMP5* and *BMPRI1A* (ALK3), and metastatic lymph nodes retain strong *BMP2*, *BMP4* and *BMP5* expression [130,131]. A recent tissue microarray of HNSCC found that the majority of samples [132] (98%) expressed *BMP2*. Furthermore, high levels of *BMP2* expression were associated with increased tumour recurrence, a finding consistent with prior studies that demonstrated that treatment of oral carcinoma cell lines with BMP2 resulted in more aggressive cell invasion and metastasis [132,133]. In addition, by using global

expressional profiles, Zhou et al. [134] found that increased *BMP2* expression was associated with increased lymph node metastasis and extracapsular spread of metastatic lymph nodes.

Activation of the BMP signalling pathway has also been demonstrated in HNSCC. In HNSCC primary specimens, *BMP4* expression and SMAD1 phosphorylation were increased, and higher levels of each were associated with poorer clinical prognosis [135]. In *in vitro* studies, stimulation of HNSCC with *BMP4* induced epithelial–mesenchymal transition (EMT), which is thought to be an important step in tumour invasion. In addition, *in vitro* knockdown of SMAD1 in Tu686 and Tu212 HNSCC cell lines inhibited EMT and migration and invasiveness of the cancer cell lines [135]. In a 3D model of cancer cell migration, suppression of *BMP4* expression by the microRNA let-7i not only altered cell morphology, but also reduced mesenchymal cell migration [136]. In addition, the same study reported elevated expression of *BMP4* and decreased expression of let-7i in the majority of HNSCC specimens, consistent with BMP signal activation [136]. Similar studies have also found that higher levels of *BMP6* and *Id1* expression in oesophageal SCC were also associated with lower 2- and 5-year survival rates [137,138], and that high *Id1* expression was an independent prognostic factor for poor prognosis in oesophageal SCC patients [139]. These studies suggest that BMP signalling is pro-tumorigenic in HNSCC and that further investigation of small molecule BMP inhibitor use is warranted, as they may offer promising primary or adjunctive treatment for HNSCC patients.

Small molecule BMP inhibitors

First generation small molecule BMP inhibitors: dorsomorphin, LDN-193189

In zebrafish embryos, BMP signalling functions to establish dorsoventral axis by specifying the ventral fates. In an *in vivo* zebrafish screen, dorsomorphin (DM; Figure 3; Table 3) was found to recapitulate in a dose-dependent manner the dorsalized phenotype associated with mutations in the BMP pathway [140]. DM, a pyrazolopyrimidine derivative (6-[4-(2-piperidin-1-ylethoxy)phenyl]-3-pyridin-4-ylpyrazolo[1,5-a]pyrimidine), was the first identified small molecule inhibitor of the BMP signalling pathway that had minimal effects on TGF- β and activin. For example, DM inhibited BMP-induced phosphorylation of SMAD1/5/8, but had no effect on MAPK p38 activation or TGF- β 1-induced activation of SMAD2. Furthermore, whereas DM inhibited the activity of constitutively active ALK2/3/6, BMP type I receptors, it had no effect on the activity of ALK4/5/7, the TGF- β and activin type I receptors [140]. In addition, DM did not cause cyclopia in zebrafish, a phenotype associated with disruption of TGF- β and activin signalling [141].

The potential pharmacological use of DM, however, has been tempered by its moderate inhibition of BMP signalling in cells ($IC_{50} \sim 0.5 \mu M$) and metabolic instability in mouse liver ($t_{1/2} = 10.4$ min) as well as its significant off-target effects [27]. Because DM is structurally homologous with known inhibitors of the vascular endothelial growth factor type II receptor, (VEGF-2/KDR), DM, not surprisingly, significantly inhibits not only KDR but also platelet-derived growth factor receptor- β (PDGFR- β) signalling at a potency level close to that of BMP ($IC_{50} \sim 0.8 \mu M$) [142].

A follow-up structure–activity relationship (SAR) studies led to the identification of a DM derivative, LDN-193189 (4-(6-(4-(piperazin-1-yl)phenyl)pyrazolo[1,5-a]pyrimidin-3-yl)quinoline hydrochloride; Figure 3; Table 3) [27]. DM and LDN-193189 share the same pyrazolo[1,5-a]pyrimidine scaffold but LDN-193189 is more potent and selective for BMP signalling (~100 fold) and also more metabolically stable. LDN-193189 also has less activity against PDGFR- β (~10 fold). Moreover, in a mouse model of FOP, LDN-193189 dramatically reduced heterotopic ossification [140]. However, *in vivo* assays using DM and LDN-193189 in zebrafish resulted in early lethality at higher concentrations, presumably from simultaneous dual inhibition of BMP and TGF- β /nodal signalling during the critical stages of early embryonic development [143].

Second and third-generation small molecule BMP inhibitors

A large *in vivo* SAR study of dorsomorphin analogues identified DMH1 (4-[6-[4-(1-methylethoxy) phenyl]pyrazolo[1,5-a]pyrimidin-3-yl]-quinoline), DMH2 (4-(2-(4-(3-(quinolin-4-yl)pyrazolo[1,5-a]pyrimidin-6-yl)phenoxy)ethyl)morpholine) and DMH3 (N,N-dimethyl-3-(4-(3-(quinolin-4-yl)pyrazolo[1,5-a]pyrimidin-6-yl)phenoxy)propan-1-amine) as synthetic compounds with the highest selectivity for BMP pathway based on specificity for causing dorsalization without additional effects (Figure 3; Table 3) [143]. In comparison with DM and LDN-193189, DMH1 is very selective for ALK2 with no discernable activity against ALK5 (TGFR-1), AMPK, KDR (VEGFR2), PDGFR-beta, or BMP-4 induced p38 MAPK phosphorylation [143]. A further refinement led to the synthesis of ML347 (5-(6-(4-methoxyphenyl)pyrazolo[1,5-a]pyrimidin-3-yl)quinolone; Figure 3; Table 3), a potent inhibitor (IC₅₀ = 152 nM) that can discriminate between type I BMP receptors: >300-fold selectivity for ALK2 compared with ALK3 [144]. A recent independent screen also identified a highly selective 2-aminopyridine compound K02288 (3-[6-amino-5-(3,4,5-trimethoxy-phenyl)-pyridin-3-yl]-phenol; Figure 3; Table 3) that inhibits BMP-stimulated phosphorylation of SMAD1/5/8 without affecting TGF- β signalling [145]. Another novel compound, LDN-214117 (1-(4-(6-methyl-5-(3,4,5-trimethoxyphenyl)pyridin-3-yl)phenyl)piperazine; Figure 3; Table 3) has a higher degree of selectivity for ALK2 than LDN-193189 or K02288 and a lower cytotoxic profile [146].

The use of small molecule BMP inhibitors in preclinical models of cancer therapy

The emergence of small molecule BMP inhibitors as *in vitro* and *in vivo* chemical tools has prompted recent studies on the therapeutic potential of pharmacological inhibition of BMP signalling for various cancers. Although the number of cancer types in which BMP inhibitors show therapeutic potential is increasing, the first such studies have focused on the effects of BMP inhibitors on lung cancer, epithelial ovarian cancer and breast cancer.

Lung cancer

The application of small molecule BMP inhibitors to lung cancer therapy has been rigorously studied and shown to be effective in suppressing lung cancer tumorigenesis *in vitro* and *in vivo*. In the highly malignant A549 lung adenocarcinoma cells, which have increased expression of BMP2, LDN-193189 reduced cell growth and promoted cell death

[147]. In a separate study, LDN-193189, along with DMH1 and DMH2, decreased cell growth, proliferation and clonogenicity of both the A549 and H1299 cell lines [64]. In addition, DMH2, which is highly selective for ALK2, was the most potent inhibitor of BMP signalling in both the A549 and H1299 cell lines and resulted in significant attenuation of cell growth and Id expression [64]. DMH2 also significantly inhibited cancer cell growth of A549 and H1229 cell lines expressing Oct4 or Nestin, stem cell markers that promote cell survival and are associated with increased chemo-resistance [148]. More recently, DMH1, which is more selective for ALK-2 than DMH2, significantly reduced tumour growth in a mouse xenograft model of NSCLC [149].

Epithelial ovarian cancer

DM and LDN offer promising adjuvant therapy for chemo-resistant epithelial ovarian cancer (EOC). DM and LDN-193189 increased cancer survival and decreased cell viability in a dose-dependent manner in a mouse intraperitoneal xenograft model of EOC [150]. Furthermore, in cisplatin (CP)-sensitive and CP-resistant EOC cell lines, DM and LDN-193189 worked synergistically through different mechanisms to resensitize platinum-resistant EOC cells to platinum agents-DM induced cell autophagy whereas LDN-193189 induced cell apoptosis [150]. Similarly, the more selective DMH1 has been recently demonstrated to be an effective neoadjuvant to cisplatin. Together with cisplatin, DMH1 reduced ovarian sphere growth and enhanced cisplatin response of the chemo-resistant cell lines OVCAR8 and NCI-RES [151].

Breast cancer

Recent studies have suggested that inhibition of BMP signalling in breast cancer can provide therapeutic benefit both *in vitro* and *in vivo*. In A17 breast cancer cells, DM reduced cell proliferation and reduced expression of EMT markers Vimentin, Snail and Slug [152]. In mammary epithelial cell lines and primary murine tumour cells, LDN-193189 inhibited clonogenicity and cell migration [153]. In a more recent study using a murine model of breast cancer, DMH1 reduced not only primary tumour burden but also lung metastasis [115]. DMH-1 treated tumours also had reduced lymphatic vessel growth and decreased expression of EMT markers, such as Snail and Twist [115].

Taken together, these studies on lung, epithelial ovarian and breast cancer suggest that small molecule BMP inhibitors may represent a novel class of therapeutic agents. However, further studies are necessary to investigate both potential off-target and clinical side effects.

Concluding remarks

Although the role of BMP signalling in cancer has been less studied compared with the closely related TGF- β pathway, it is now clear that BMP signalling plays many important roles in cancer biology. In many cancers, BMPs appear to have multifunctional and often seemingly opposing roles in promoting or inhibiting cancer, prompting additional studies to further clarify the BMP signalling context. Different BMP ligands and receptors as well as the canonical SMAD-dependent and SMAD-independent pathways probably play distinct roles in different cancers and different cancer processes. In addition, BMP signalling affects

not just cancer cells directly but also the surrounding tumour microenvironment, including tumour microvasculature and immune cells. Against the backdrop of complex cancer biology, the discovery of dorsomorphin and subsequent generations of BMP small molecule inhibitors has spurred a growing number of studies that suggest that BMP signalling may indeed be a promising therapeutic target for a variety of cancers. For instance, using DMH1, the most selective known inhibitor of ALK1, ALK2 and ALK3, we showed that a systemic inhibition of BMP signalling not only reduced primary tumour burden in a spontaneous mouse model of metastatic breast cancer, but also reduced metastasis via its effects on the stromal compartments of fibroblasts, lymphatic vessel drainage of tumours and polarization of the immune system [154]. Development of newer and more selective compounds with improved pharmacokinetic characteristics will not only advance mechanistic studies of cancers *in vivo* but also lead to potentially effective cancer therapies.

From the translational medicine perspective, the complexity of BMP signalling will require a detailed understanding of the involvement of specific BMP signalling components in individual tumours. The status of BMP signal activation, and its effects on cancer cells and tumour microenvironment can be evaluated in a patient's primary tumours as well as recurrent or metastatic tumours in order to formulate individualized therapeutic strategy. Such analysis may also affect the choice of specific BMP inhibitors, with varying selectivity, should these agents become available in the clinic. From a mechanistic standpoint, we have focused on developing small molecule inhibitors that are exquisitely selective for the type I BMP receptors, even at the level of subtype-specificity, yet some cancers may benefit from a 'pan' BMP inhibitor or off-target effects on other pathways. In the near future, BMP inhibitors may be useful as stand-alone agent, as an adjuvant to the standard of care, or as a component of a cocktail of successful targeted therapies, including cancer immunotherapies. In conclusion, a more detailed molecular understanding of the role of BMP signalling in a variety of cancer contexts may herald an era of new class of pharmaceuticals that target BMP signalling in the armamentarium of precision medicines in oncology.

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Abbreviations

ALK1	activin receptor like kinase-1
AMKL	acute megakaryoblastic leukaemia
BMP	bone morphogenetic protein
BPH	benign prostatic hyperplasia
CA-MSC	carcinoma-associated MSC
CML	chronic myelogenous leukaemia

CRC	colorectal cancer
DIPG	diffuse intrinsic pontine glioma
DM	dorsomorphin
EOC	epithelial ovarian cancer
FOP	fibrodysplasia ossificans progressiva
HNSCC	head and neck squamous cell carcinoma
HSC	hematopoietic stem cell
LIMK	LIM domain kinase
mTOR	mammalian target of rapamycin
MAPK	mitogen-activated protein kinase
MFH	malignant fibrous histiosarcoma
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
NSCLC	non-small cell lung cancer
PDAC	pancreatic ductal adenocarcinoma
PI3K	phosphoinositide 3-kinase
SAR	structure–activity relationship
SNP	single nucleotide polymorphism
TGF-β	transforming growth factor- β
TKI	tyrosine kinase inhibitor
TSG	twisted gastrulation

References

1. Urist MR. Bone: formation by autoinduction. *Science*. 1965; 150:893–899. [PubMed: 5319761]
2. Reddi AH, Huggins C. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. *Proc Natl Acad Sci USA*. 1972; 69:1601–1605. [PubMed: 4504376]
3. Xiao YT, Xiang LX, Shao JZ. Bone morphogenetic protein. *Biochem Biophys Res Commun*. 2007; 362:550–553. [PubMed: 17719560]
4. Sieber C, Kopf J, Hiepen C, Knaus P. Recent advances in BMP receptor signaling. *Cytokine Growth Factor Rev*. 2009; 20:343–355. [PubMed: 19897402]
5. Mueller TD, Nickel J. Promiscuity and specificity in BMP receptor activation. *FEBS Lett*. 2012; 586:1846–1859. [PubMed: 22710174]
6. Nohe A, Keating E, Knaus P, Petersen NO. Signal transduction of bone morphogenetic protein receptors. *Cell Signal*. 2004; 16:291–299. [PubMed: 14687659]

7. Little SC, Mullins MC. Bone morphogenetic protein heterodimers assemble heteromeric type I receptor complexes to pattern the dorsoventral axis. *Nat Cell Biol.* 2009; 11:637–643. [PubMed: 19377468]
8. Yadin D, Knaus P, Mueller TD. Structural insights into BMP receptors: specificity, activation and inhibition. *Cytokine Growth Factor Rev.* 2016; 27:13–34. [PubMed: 26690041]
9. Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily. *Science.* 2002; 296:1646–1647. [PubMed: 12040180]
10. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature.* 2003; 425:577–584. [PubMed: 14534577]
11. Avsian-Kretchmer O, Hsueh AJW. Comparative genomic analysis of the eight-membered ring cystine knot-containing bone morphogenetic protein antagonists. *Mol Endocrinol.* 2004; 18:1–12. [PubMed: 14525956]
12. Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-beta/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. *Bone Res.* 2015; 3:15005. [PubMed: 26273537]
13. Scoville DH, Sato T, He XC, Li L. Current view: intestinal stem cells and signaling. *Gastroenterology.* 2008; 134:849–864. [PubMed: 18325394]
14. Voorneveld PW, Kodach LL, Jacobs RJ, Liv N, Zonneville AC, Hoogenboom JP, Biemond I, Verspaget HW, Hommes DW, de Rooij K, et al. Loss of SMAD4 alters BMP signaling to promote colorectal cancer cell metastasis via activation of Rho and ROCK. *Gastroenterology.* 2014; 147:196–208, e13. [PubMed: 24704720]
15. Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell.* 2000; 103:295–309. [PubMed: 11057902]
16. Jass JR, Williams CB, Bussey HJR, Morson BC. Juvenile polyposis – a precancerous condition. *Histopathology.* 1988; 13:619–630. [PubMed: 2853131]
17. van Hattem WA, Brosens LA, de Leng WW, Morsink FH, Lens S, Carvalho R, Giardiello FM, Offerhaus GJ. Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. *Gut.* 2008; 57:623–627. [PubMed: 18178612]
18. Calva-Cerqueira D, Chinnathambi S, Pechman B, Bair J, Larsen-Haidle J, Howe JR. The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. *Clin Genet.* 2009; 75:79–85. [PubMed: 18823382]
19. Woodford-Richens KL, Rowan AJ, Poulsom R, Bevan S, Salovaara R, Aaltonen LA, Houlston RS, Wright NA, Tomlinson IP. Comprehensive analysis of SMAD4 mutations and protein expression in juvenile polyposis: evidence for a distinct genetic pathway and polyp morphology in SMAD4 mutation carriers. *Am J Pathol.* 2001; 159:1293–1300. [PubMed: 11583957]
20. Haramis AP, Begthel H, van den Born M, van Es J, Jonkheer S, Offerhaus GJ, Clevers H. *De novo* crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science.* 2004; 303(5664):1684–6. [PubMed: 15017003]
21. He XC, Zhang J, Tong WG, Tawfik O, Ross J, Scoville DH, Tian Q, Zeng X, He X, Wiedemann LM, et al. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat Genet.* 2004; 36:1117–1121. [PubMed: 15378062]
22. Hardwick JC, Van Den Brink GR, Bleuming SA, Ballester I, Van Den Brande JM, Keller JJ, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP. Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology.* 2004; 126:111–121. [PubMed: 14699493]
23. Beck SE, Jung BH, Fiorino A, Gomez J, Rosario ED, Cabrera BL, Huang SC, Chow JY, Carethers JM. Bone morphogenetic protein signaling and growth suppression in colon cancer. *Am J Physiol Gastrointest Liver Physiol.* 2006; 291:G135–G145. [PubMed: 16769811]
24. Deng H, Makizumi R, Ravikumar TS, Dong H, Yang W, Yang WL. Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. *Exp Cell Res.* 2007; 313:1033–1044. [PubMed: 17275810]
25. Kodach LL, Wiercinska E, de Miranda NF, Bleuming SA, Musler AR, Peppelenbosch MP, Dekker E, van den Brink GR, van Noesel CJ, Morreau H, et al. The bone morphogenetic protein pathway

- is inactivated in the majority of sporadic colorectal cancers. *Gastroenterology*. 2008; 134:1332–1341. [PubMed: 18471510]
26. Kodach LL, Bleuming SA, Musler AR, Peppelenbosch MP, Hommes DW, van den Brink GR, van Noesel CJ, Offerhaus GJ, Hardwick JC. The bone morphogenetic protein pathway is active in human colon adenomas and inactivated in colorectal cancer. *Cancer*. 2008; 112:300–306. [PubMed: 18008360]
 27. Cuny GD, Yu PB, Laha JK, Xing X, Liu JF, Lai CS, Deng DY, Sachidanandan C, Bloch KD, Peterson RT. Structure-activity relationship study of bone morphogenetic protein (BMP) signaling inhibitors. *Bioorg Med Chem Lett*. 2008; 18:4388–4392. [PubMed: 18621530]
 28. Motoyama K, Tanaka F, Kosaka Y, Mimori K, Uetake H, Inoue H, Sugihara K, Mori M. Clinical significance of BMP7 in human colorectal cancer. *Ann Surg Oncol*. 2008; 15:1530–1537. [PubMed: 18259822]
 29. Athale UH, Razzouk BI, Raimondi SC, Tong X, Behm FG, Head DR, Srivastava DK, Rubnitz JE, Bowman L, Pui CH, Ribeiro RC. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. *Blood*. 2001; 97:3727–3732. [PubMed: 11389009]
 30. Barnard DR, Alonzo TA, Gerbing RB, Lange B, Woods WG. Comparison of childhood myelodysplastic syndrome, AML FAB M6 or M7, CCG 2891: report from the Children's Oncology Group. *Pediatr Blood Cancer*. 2007; 49:17–22. [PubMed: 16856158]
 31. Gassmann W, Löffler H. Acute megakaryoblastic leukemia. *Leuk Lymph*. 1995; 18(s1):69–73.
 32. Ge Y, Jensen TL, Stout ML, Flatley RM, Grohar PJ, Ravindranath Y, Matherly LH, Taub JW. The role of cytidine deaminase and GATA1 mutations in the increased cytosine arabinoside sensitivity of down syndrome myeloblasts and leukemia cell lines. *Cancer Res*. 2004; 64:728–735. [PubMed: 14744791]
 33. Gruber TA, Gedman AL, Zhang J, Koss CS, Marada S, Ta HQ, Chen SC, Su X, Ogden SK, Dang J, et al. An inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. *Cancer Cell*. 2012; 22:683–697. [PubMed: 23153540]
 34. Chyla BJ, Moreno-Miralles I, Steapleton MA, Thompson MA, Bhaskara S, Engel M, Hiebert SW. Deletion of Mtg16, a target of t(16;21), alters hematopoietic progenitor cell proliferation and lineage allocation. *Mol Cell Biol*. 2008; 28:6234–6247. [PubMed: 18710942]
 35. Zhang F, Nakanishi G, Kurebayashi S, Yoshino K, Perantoni A, Kim YS, Jetten AM. Characterization of Glis2, a novel gene encoding a Gli-related, Kruppel-like transcription factor with transactivation and repressor functions. Roles in kidney development and neurogenesis. *J Biol Chem*. 2002; 277:10139–10149. [PubMed: 11741991]
 36. Attanasio M, Uhlenhaut NH, Sousa VH, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, et al. Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat Genet*. 2007; 39:1018–1024. [PubMed: 17618285]
 37. Sander V, Eivers E, Choi RH, De Robertis EM. Drosophila Smad2 opposes Mad signaling during wing vein development. *PLoS One*. 2010; 5:e10383. [PubMed: 20442782]
 38. Jeanpierre S, Nicolini FE, Kaniewski B, Dumontet C, Rimokh R, Puisieux A, Maguer-Satta V. BMP4 regulation of human megakaryocytic differentiation is involved in thrombopoietin signaling. *Blood*. 2008; 112:3154–3163. [PubMed: 18664625]
 39. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med*. 1999; 340:1330–1340. [PubMed: 10219069]
 40. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001; 344:1031–1037. [PubMed: 11287972]
 41. Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, Barow M, Mountford JC, Holyoake TL. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006; 107:4532–4539. [PubMed: 16469872]

42. Toofan P, Irvine D, Hopcroft L, Copland M, Wheadon H. The role of the bone morphogenetic proteins in leukaemic stem cell persistence. *Biochem Soc Trans.* 2014; 42:809–815. [PubMed: 25109962]
43. Laperrousaz B, Jeanpierre S, Sagorny K, Voeltzel T, Ramas S, Kaniewski B, Ffrench M, Salesse S, Nicolini FE, Maguer-Satta V. Primitive CML cell expansion relies on abnormal levels of BMPs provided by the niche and on BMPRIb overexpression. *Blood.* 2013; 122:3767–3777. [PubMed: 24100446]
44. Topic I, Ilic M, Ivcevic S, Kovacic N, Marusic A, Kusec R, Grcevic D. Bone morphogenetic proteins regulate differentiation of human promyelocytic leukemia cells. *Leuk Res.* 2013; 37:705–712. [PubMed: 23528261]
45. Raymond A, Liu B, Liang H, Wei C, Guindani M, Lu Y, Liang S, St John LS, Molldrem J, Nagarajan L. A role for BMP-induced homeobox gene MIXL1 in acute myelogenous leukemia and identification of type I BMP receptor as a potential target for therapy. *Oncotarget.* 2014; 5:12675–12693. [PubMed: 25544748]
46. Jansen MHA, van Vuurden DG, Vandertop WP, Kaspers GJL. Diffuse intrinsic pontine gliomas: A systematic update on clinical trials and biology. *Cancer Treat Rev.* 2012; 38:27–35. [PubMed: 21764221]
47. Jones C, Perryman L, Hargrave D. Paediatric and adult malignant glioma: close relatives or distant cousins. *Nat Rev Clin Oncol.* 2012; 9:400–413. [PubMed: 22641364]
48. Furuta Y, Piston DW, Hogan BL. Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development.* 1997; 124:2203–2212. [PubMed: 9187146]
49. Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, Zhu X, Qu C, Chen X, Zhang J, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet.* 2014; 46:444–450. [PubMed: 24705251]
50. Buczkowicz P, Hoeman C, Rakopoulos P, Pajovic S, Letourneau L, Dzamba M, Morrison A, Lewis P, Bouffet E, Bartels U, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet.* 2014; 46:451–456. [PubMed: 24705254]
51. Taylor KR, Mackay A, Truffaux N, Butterfield YS, Morozova O, Philippe C, Castel D, Grasso CS, Vinci M, Carvalho D, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat Genet.* 2014; 46:457–461. [PubMed: 24705252]
52. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, Nikbakht H, Gerges N, Fiset P-O, Bechet D, Fauray D, De Jay N, Ramkissoon LA, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet.* 2014; 46:462–466. [PubMed: 24705250]
53. Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho TJ, Choi IH, Connor JM, Delai P, Glaser DL, Le Merrer M, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat Genet.* 2006; 38:525–527. [PubMed: 16642017]
54. Shen Q, Little SC, Xu M, Haupt J, Ast C, Katagiri T, Mundlos S, Seemann P, Kaplan FS, Mullins MC, Shore EM. The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization. *J Clin Invest.* 2009; 119:3462–3472. [PubMed: 19855136]
55. Hover LD, Owens P, Munden AL, Wang J, Chambless LB, Hopkins CR, Hong CC, Moses HL, Abel TW. Bone morphogenetic protein signaling promotes tumorigenesis in a murine model of high-grade glioma. *Neuro Oncol.* 2016; 18:928–938. [PubMed: 26683138]
56. Jiang S, Zhang S, Langenfeld J, Lo SC, Rogers MB. Mycoplasma infection transforms normal lung cells and induces bone morphogenetic protein 2 expression by post-transcriptional mechanisms. *J Cell Biochem.* 2008; 104:580–594. [PubMed: 18059017]
57. Langenfeld EM, Kong Y, Langenfeld J. Bone morphogenetic protein 2 stimulation of tumor growth involves the activation of Smad-1/5. *Oncogene.* 2006; 25:685–692. [PubMed: 16247476]
58. Fei ZH, Yao CY, Yang XL, Huang XE, Ma SL. Serum BMP-2 up-regulation as an indicator of poor survival in advanced non-small cell lung cancer patients. *Asian Pac J Cancer Prev.* 2013; 14:5293–5299. [PubMed: 24175816]

59. Choi YJ, Kim ST, Park KH, Oh SC, Seo JH, Shin SW, Kim JS, Kim YH. The serum bone morphogenetic protein-2 level in non-small-cell lung cancer patients. *Med Oncol.* 2012; 29:582–588. [PubMed: 21327739]
60. Chu H, Luo H, Wang H, Chen X, Li P, Bai Y, Zhang F, Cheng R, Chen S, Wang Y, et al. Silencing BMP-2 expression inhibits A549 and H460 cell proliferation and migration. *Diagn Pathol.* 2014; 9:123. [PubMed: 24946687]
61. Lin M, Stewart DJ, Spitz MR, Hildebrandt MA, Lu C, Lin J, Gu J, Huang M, Lippman SM, Wu X. Genetic variations in the transforming growth factor-beta pathway as predictors of survival in advanced non-small cell lung cancer. *Carcinogenesis.* 2011; 32:1050–1056. [PubMed: 21515830]
62. Langenfeld EM, Calvano SE, Abou-Nukta F, Lowry SF, Amenta P, Langenfeld J. The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. *Carcinogenesis.* 2003; 24:1445–1454. [PubMed: 12819188]
63. Langenfeld EM, Langenfeld J. Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. *Mol Cancer Res.* 2004; 2:141–149. [PubMed: 15037653]
64. Langenfeld E, Hong CC, Lanke G, Langenfeld J. Bone morphogenetic protein type I receptor antagonists decrease growth and induce cell death of lung cancer cell lines. *PLoS One.* 2013; 8:e61256. [PubMed: 23593444]
65. Warshaw AL, Fernandez-del Castillo C. Pancreatic carcinoma. *N Engl J Med.* 1992; 326:455–465. [PubMed: 1732772]
66. Gordon KJ, Kirkbride KC, How T, Blobe GC. Bone morphogenetic proteins induce pancreatic cancer cell invasiveness through a Smad1-dependent mechanism that involves matrix metalloproteinase-2. *Carcinogenesis.* 2009; 30:238–248. [PubMed: 19056927]
67. Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science.* 1996; 271:350–353. [PubMed: 8553070]
68. Rozenblum E, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res.* 1997; 57:1731–174. [PubMed: 9135016]
69. Tascilar M, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res.* 2001; 7:4115–4121. [PubMed: 11751510]
70. Wilentz RE, Iacobuzio-Donahue CA, Argani P, McCarthy DM, Parsons JL, Yeo CJ, Kern SE, Hruban RH. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res.* 2000; 60:2002–2006. [PubMed: 10766191]
71. Duda DG, Sunamura M, Lefter LP, Furukawa T, Yokoyama T, Yatsuoka T, Abe T, Inoue H, Motoi F, Egawa S, et al. Restoration of SMAD4 by gene therapy reverses the invasive phenotype in pancreatic adenocarcinoma cells. *Oncogene.* 2003; 22:6857–6864. [PubMed: 14534532]
72. Voorneveld PW, Stache V, Jacobs RJ, Smolders E, Sitters AI, Liesker A, Korkmaz KS, Lam SM, De Miranda NF, Morreau H, et al. Reduced expression of bone morphogenetic protein receptor IA in pancreatic cancer is associated with a poor prognosis. *Br J Cancer.* 2013; 109:1805–1812. [PubMed: 23969729]
73. Brucks JA. Ovarian cancer. The most lethal gynecologic malignancy. *Nurs Clin North Am.* 1992; 27:835–845. [PubMed: 1448359]
74. Sherman-Baust CA, Becker KG, Wood WH Iii, Zhang Y, Morin PJ. Gene expression and pathway analysis of ovarian cancer cells selected for resistance to cisplatin, paclitaxel, or doxorubicin. *J Ovarian Res.* 2011; 4:21. [PubMed: 22141344]
75. Shepherd TG, Theriault BL, Nachtigal MW. Autocrine BMP4 signalling regulates ID3 proto-oncogene expression in human ovarian cancer cells. *Gene.* 2008; 414:95–105. [PubMed: 18372118]
76. Shepherd TG, Nachtigal MW. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. *Endocrinology.* 2003; 144:3306–3314. [PubMed: 12865307]

77. Le Page C, Puiffe ML, Meunier L, Zietarska M, de Ladurantaye M, Tonin PN, Provencher D, Mes-Masson AM. BMP-2 signaling in ovarian cancer and its association with poor prognosis. *J Ovarian Res.* 2009; 2:4. [PubMed: 19366455]
78. Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F. Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther.* 2008; 15:730–738. [PubMed: 18401438]
79. McLean K, Gong Y, Choi Y, Deng N, Yang K, Bai S, Cabrera L, Keller E, McCauley L, Cho KR, Buckanovich RJ. Human ovarian carcinoma-associated mesenchymal stem cells regulate cancer stem cells and tumorigenesis via altered BMP production. *J Clin Invest.* 2011; 121:3206–3219. [PubMed: 21737876]
80. Peng J, Yoshioka Y, Mandai M, Matsumura N, Baba T, Yamaguchi K, Hamanishi J, Kharma B, Murakami R, Abiko K, et al. The BMP signaling pathway leads to enhanced proliferation in serous ovarian cancer-a potential therapeutic target. *Mol Carcinog.* 2016; 55:335–345. [PubMed: 25663289]
81. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer.* 2009; 115:1531–1543. [PubMed: 19197972]
82. Mohseny AB, Cai Y, Kuijjer M, Xiao W, van den Akker B, de Andrea CE, Jacobs R, ten Dijke P, Hogendoorn PC, Cleton-Jansen AM. The activities of Smad and Gli mediated signalling pathways in high-grade conventional osteosarcoma. *Eur J Cancer.* 2012; 48:3429–3438. [PubMed: 22868198]
83. Yoshikawa H, Rettig WJ, Lane JM, Takaoka K, Alderman E, Rup B, Rosen V, Healey JH, Huvos AG, Garin-Chesa P. Immunohistochemical detection of bone morphogenetic proteins in bone and soft-tissue sarcomas. *Cancer.* 1994; 74:842–847. [PubMed: 8039112]
84. Sulzbacher I, Birner P, Trieb K, Pichlbauer E, Lang S. The expression of bone morphogenetic proteins in osteosarcoma and its relevance as a prognostic parameter. *J Clin Pathol.* 2002; 55:381–385. [PubMed: 11986346]
85. Yoshikawa H, Takaoka K, Masuhara K, Ono K, Sakamoto Y. Prognostic significance of bone morphogenetic activity in osteosarcoma tissue. *Cancer.* 1988; 61:569–573. [PubMed: 3422172]
86. Guo W, Gorlick R, Ladanyi M, Meyers PA, Huvos AG, Bertino JR, Healey JH. Expression of bone morphogenetic proteins and receptors in sarcomas. *Clin Orthop Relat Res.* 1999; 365:175–183.
87. Yoshikawa H, Nakase T, Myoui A, Ueda T. Bone morphogenetic proteins in bone tumors. *J Orthop Sci.* 2004; 9:334–340. [PubMed: 15168194]
88. Cong Y, Li CJ, Zhao JN, Liu XZ, Shi X. Associations of polymorphisms in the bone morphogenetic protein-2 gene with risk and prognosis of osteosarcoma in a Chinese population. *Tumour Biol.* 2015; 36:2059–2064. [PubMed: 25391427]
89. Luo X, Chen J, Song WX, Tang N, Luo J, Deng ZL, Sharff KA, He G, Bi Y, He BC, et al. Osteogenic BMPs promote tumor growth of human osteosarcomas that harbor differentiation defects. *Lab Invest.* 2008; 88:1264–1277. [PubMed: 18838962]
90. Sotobori T, Ueda T, Myoui A, Yoshioka K, Nakasaki M, Yoshikawa H, Itoh K. Bone morphogenetic protein-2 promotes the haptotactic migration of murine osteoblastic and osteosarcoma cells by enhancing incorporation of integrin beta1 into lipid rafts. *Exp Cell Res.* 2006; 312:3927–3938. [PubMed: 17022972]
91. Kakonen SM, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. *Cancer.* 2003; 97(3 Suppl):834–839. [PubMed: 12548583]
92. Bentley H, Hamdy FC, Hart KA, Seid JM, Williams JL, Johnstone D, Russell RG. Expression of bone morphogenetic proteins in human prostatic adenocarcinoma and benign prostatic hyperplasia. *Br J Cancer.* 1992; 66:1159–1163. [PubMed: 1280991]
93. Autzen P, Robson CN, Bjartell A, Malcolm AJ, Johnson MI, Neal DE, Hamdy FC. Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. *Br J Cancer.* 1998; 78:1219–1223. [PubMed: 9820184]
94. Hamdy FC, Autzen P, Robinson MC, Horne CH, Neal DE, Robson CN. Immunolocalization and messenger RNA expression of bone morphogenetic protein-6 in human benign and malignant prostatic tissue. *Cancer Res.* 1997; 57:4427–4431. [PubMed: 9331107]

95. Barnes J, Anthony CT, Wall N, Steiner MS. Bone morphogenetic protein-6 expression in normal and malignant prostate. *World J Urol.* 1995; 13:337–343. [PubMed: 9116752]
96. Darby S, Cross SS, Brown NJ, Hamdy FC, Robson CN. BMP-6 over-expression in prostate cancer is associated with increased Id-1 protein and a more invasive phenotype. *J Pathol.* 2008; 214:394–404. [PubMed: 18072288]
97. Dai J, Keller J, Zhang J, Lu Y, Yao Z, Keller ET. Bone morphogenetic protein-6 promotes osteoblastic prostate cancer bone metastases through a dual mechanism. *Cancer Res.* 2005; 65:8274–8285. [PubMed: 16166304]
98. Kim IY, Lee DH, Ahn HJ, Tokunaga H, Song W, Devereaux LM, Jin D, Sampath TK, Morton RA. Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. *Cancer Res.* 2000; 60:2840–2844. [PubMed: 10850425]
99. Kim IY, Lee DH, Lee DK, Ahn HJ, Kim MM, Kim SJ, Morton RA. Loss of expression of bone morphogenetic protein receptor type II in human prostate cancer cells. *Oncogene.* 2004; 23:7651–7659. [PubMed: 15354178]
100. Horvath LG, Henshall SM, Kench JG, Turner JJ, Golovsky D, Brenner PC, O'Neill GF, Kooner R, Stricker PD, Grygiel JJ, Sutherland RL. Loss of BMP2, Smad8, and Smad4 expression in prostate cancer progression. *Prostate.* 2004; 59:234–242. [PubMed: 15042598]
101. Feeley BT, Krenek L, Liu N, Hsu WK, Gamradt SC, Schwarz EM, Huard J, Lieberman JR. Overexpression of noggin inhibits BMP-mediated growth of osteolytic prostate cancer lesions. *Bone.* 2006; 38:154–166. [PubMed: 16126463]
102. Yang S, Zhong C, Frenkel B, Reddi AH, Roy-Burman P. Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. *Cancer Res.* 2005; 65:5769–5777. [PubMed: 15994952]
103. Ghosh-Choudhury N, Ghosh-Choudhury G, Celeste A, Ghosh PM, Moyer M, Abboud SL, Kreisberg J. Bone morphogenetic protein-2 induces cyclin kinase inhibitor p21 and hypophosphorylation of retinoblastoma protein in estradiol-treated MCF-7 human breast cancer cells. *Biochim Biophys Acta.* 2000; 1497:186–196. [PubMed: 10903423]
104. Waite KA, Eng C. BMP2 exposure results in decreased PTEN protein degradation and increased PTEN levels. *Hum Mol Genet.* 2003; 12:679–684. [PubMed: 12620973]
105. Soda H, Raymond E, Sharma S, Lawrence R, Cerna C, Gomez L, Timony GA, Von Hoff DD, Izbicka E. Antiproliferative effects of recombinant human bone morphogenetic protein-2 on human tumor colony-forming units. *Anticancer Drugs.* 1998; 9:327–331. [PubMed: 9635923]
106. Clement JH, Raida M, Sanger J, Bicknell R, Liu J, Naumann A, Geyer A, Waldau A, Hortschansky P, Schmidt A, et al. Bone morphogenetic protein 2 (BMP-2) induces *in vitro* invasion and *in vivo* hormone independent growth of breast carcinoma cells. *Int J Oncol.* 2005; 27:401–407. [PubMed: 16010421]
107. Raida M, Clement JH, Ameri K, Han C, Leek RD, Harris AL. Expression of bone morphogenetic protein 2 in breast cancer cells inhibits hypoxic cell death. *Int J Oncol.* 2005; 26:1465–1470. [PubMed: 15870857]
108. Scherberich A, Tucker RP, Degen M, Brown-Luedi M, Andres A-C, Chiquet-Ehrismann R. Tenascin-W is found in malignant mammary tumors, promotes alpha8 integrin-dependent motility and requires p38MAPK activity for BMP-2 and TNF-alpha induced expression *in vitro*. *Oncogene.* 2004; 24:1525–1532.
109. Liu F, Bloch N, Bhushan KR, De Grand AM, Tanaka E, Solazzo S, Mertyna PM, Goldberg N, Frangioni JV, Lenkinski RE. Humoral bone morphogenetic protein 2 is sufficient for inducing breast cancer microcalcification. *Mol Imaging.* 2008; 7:175–186. [PubMed: 19123988]
110. Liu F, Misra P, Lunsford EP, Vannah JT, Liu Y, Lenkinski RE, Frangioni JV. A dose- and time-controllable syngeneic animal model of breast cancer microcalcification. *Breast Cancer Res Treat.* 2010; 122:87–94. [PubMed: 19760034]
111. Alarmo EL, Kuukasjarvi T, Karhu R, Kallioniemi A. A comprehensive expression survey of bone morphogenetic proteins in breast cancer highlights the importance of BMP4 and BMP7. *Breast Cancer Res Treat.* 2007; 103:239–246. [PubMed: 17004110]

112. Shon SK, Kim A, Kim JY, Kim KI, Yang Y, Lim JS. Bone morphogenetic protein-4 induced by NDRG2 expression inhibits MMP-9 activity in breast cancer cells. *Biochem Biophys Res Commun.* 2009; 385:198–203. [PubMed: 19450561]
113. Ketolainen JM, Alarmo EL, Tuominen VJ, Kallioniemi A. Parallel inhibition of cell growth and induction of cell migration and invasion in breast cancer cells by bone morphogenetic protein 4. *Breast Cancer Res Treat.* 2010; 124:377–386. [PubMed: 20182795]
114. Owens P, Polikowsky H, Pickup MW, Gorska AE, Jovanovic B, Shaw AK, Novitskiy SV, Hong CC, Moses HL. Bone morphogenetic proteins stimulate mammary fibroblasts to promote mammary carcinoma cell invasion. *PLoS One.* 2013; 8:e67533. [PubMed: 23840733]
115. Owens P, Pickup MW, Novitskiy SV, Giltane JM, Gorska AE, Hopkins CR, Hong CC, Moses HL. Inhibition of BMP signaling suppresses metastasis in mammary cancer. *Oncogene.* 2015; 34:2437–2449. [PubMed: 24998846]
116. Helms MW, Packeisen J, August C, Schitteck B, Boecker W, Brandt BH, Buerger H. First evidence supporting a potential role for the BMP/SMAD pathway in the progression of oestrogen receptor-positive breast cancer. *J Pathol.* 2005; 206:366–376. [PubMed: 15892165]
117. Pickup MW, Hover LD, Guo Y, Gorska AE, Chytil A, Novitskiy SV, Moses HL, Owens P. Deletion of the BMP receptor BMPRIa impairs mammary tumor formation and metastasis. *Oncotarget.* 2015; 6:22890–22904. [PubMed: 26274893]
118. Alarmo EL, Korhonen T, Kuukasjarvi T, Huhtala H, Holli K, Kallioniemi A. Bone morphogenetic protein 7 expression associates with bone metastasis in breast carcinomas. *Ann Oncol.* 2008; 19:308–314. [PubMed: 17895257]
119. Katsuno Y, Hanyu A, Kanda H, Ishikawa Y, Akiyama F, Iwase T, Ogata E, Ehata S, Miyazono K, Imamura T. Bone morphogenetic protein signaling enhances invasion and bone metastasis of breast cancer cells through Smad pathway. *Oncogene.* 2008; 27:6322–6333. [PubMed: 18663362]
120. Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. *Br J Dermatol.* 2004; 150:179–185. [PubMed: 14996086]
121. Dorsky RI, Moon RT, Raible DW. Environmental signals and cell fate specification in premigratory neural crest. *Bioessays.* 2000; 22:708–716. [PubMed: 10918301]
122. Hsu MY, Rovinsky S, Penmatcha S, Herlyn M, Muirhead D. Bone morphogenetic proteins in melanoma: angel or devil. *Cancer Metastasis Rev.* 2005; 24:251–263. [PubMed: 15986135]
123. Rothhammer T, Poser I, Soncin F, Bataille F, Moser M, Bosserhoff AK. Bone morphogenic proteins are overexpressed in malignant melanoma and promote cell invasion and migration. *Cancer Res.* 2005; 65:448–456. [PubMed: 15695386]
124. Busch C, Drews U, Garbe C, Eisele SR, Oppitz M. Neural crest cell migration of mouse B16-F1 melanoma cells transplanted into the chick embryo is inhibited by the BMP-antagonist noggin. *Int J Oncol.* 2007; 31:1367–1378. [PubMed: 17982664]
125. Rothhammer T, Bataille F, Spruss T, Eissner G, Bosserhoff AK. Functional implication of BMP4 expression on angiogenesis in malignant melanoma. *Oncogene.* 2007; 26:4158–4170. [PubMed: 17173062]
126. Rothhammer T, Braig S, Bosserhoff AK. Bone morphogenetic proteins induce expression of metalloproteinases in melanoma cells and fibroblasts. *Eur J Cancer.* 2008; 44:2526–2534. [PubMed: 18774289]
127. Prince A, Aguirre-Ghizo J, Genden E, Posner M, Sikora A. Head and neck squamous cell carcinoma: new translational therapies. *Mt Sinai J Med.* 2010; 77:684–699. [PubMed: 21105129]
128. Hatakeyama S, Satoh M, Yoshimura N, Otsu T. Immunocytochemical localization of bone morphogenetic proteins (BMPs) in salivary gland pleomorphic adenoma. *J Oral Pathol Med.* 1994; 23:232–236. [PubMed: 7519267]
129. Zhao M, Takata T, Ogawa I, Takekoshi T, Nikai H. Immunohistochemical demonstration of bone morphogenetic protein-2 and type II collagen in pleomorphic adenoma of salivary glands. *J Oral Pathol Med.* 1998; 27:293–296. [PubMed: 9725565]
130. Jin Y, Tipoe GL, Liong EC, Lau TY, Fung PC, Leung KM. Overexpression of BMP-2/4, -5 and BMPRI-IA associated with malignancy of oral epithelium. *Oral Oncol.* 2001; 37:225–233. [PubMed: 11287276]

131. Soares AF, Xavier RL, da Costa Miguel MC, de Souza LB, Pinto LP. Bone morphogenetic protein-2/4 and bone morphogenetic protein receptor type IA expression in metastatic and nonmetastatic oral squamous cell carcinoma. *Am J Otolaryngol.* 2010; 31:266–271. [PubMed: 20015767]
132. Sand JP, Kokorina NA, Zakharkin SO, Lewis JS Jr, Nussenbaum B. BMP-2 expression correlates with local failure in head and neck squamous cell carcinoma. *Otolaryngol Head Neck Surg.* 2014; 150:245–250. [PubMed: 24247005]
133. Kokorina NA, Lewis JS Jr, Zakharkin SO, Krebsbach PH, Nussenbaum B. rhBMP-2 has adverse effects on human oral carcinoma cell lines *in vivo*. *Laryngoscope.* 2012; 122:95–102. [PubMed: 21997819]
134. Zhou X, Temam S, Oh M, Pungpravat N, Huang BL, Mao L, Wong DT. Global expression-based classification of lymph node metastasis and extracapsular spread of oral tongue squamous cell carcinoma. *Neoplasia.* 2006; 8:925–932. [PubMed: 17132224]
135. Xu T, Yu CY, Sun JJ, Liu Y, Wang XW, Pi LM, Tian YQ, Zhang X. Bone morphogenetic protein-4-induced epithelial-mesenchymal transition and invasiveness through Smad1-mediated signal pathway in squamous cell carcinoma of the head and neck. *Arch Med Res.* 2011; 42:128–137. [PubMed: 21565626]
136. Yang WH, Lan HY, Tai SK, Yang MH. Repression of bone morphogenetic protein 4 by let-7i attenuates mesenchymal migration of head and neck cancer cells. *Biochem Biophys Res Commun.* 2013; 433:24–30. [PubMed: 23454123]
137. Raida M, Sarbia M, Clement JH, Adam S, Gabbert HE, Hoffken K. Expression, regulation and clinical significance of bone morphogenetic protein 6 in esophageal squamous-cell carcinoma. *Int J Cancer.* 1999; 83:38–44. [PubMed: 10449605]
138. Yuen HF, McCrudden CM, Grills C, Zhang SD, Huang YH, Chan KK, Chan YP, Wong ML, Law S, Srivastava G, et al. Combinatorial use of bone morphogenetic protein 6, noggin and SOST significantly predicts cancer progression. *Cancer Sci.* 2012; 103:1145–1154. [PubMed: 22364398]
139. Yuen HF, Chan YP, Chan KK, Chu YY, Wong ML, Law SY, Srivastava G, Wong YC, Wang X, Chan KW. Id-1 and Id-2 are markers for metastasis and prognosis in oesophageal squamous cell carcinoma. *Br J Cancer.* 2007; 97:1409–1415. [PubMed: 18000500]
140. Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, Lin HY, Bloch KD, Peterson RT. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol.* 2008; 4:33–41. [PubMed: 18026094]
141. Sampath K, Rubinstein AL, Cheng AM, Liang JO, Fekany K, Solnica-Krezel L, Korzh V, Halpern ME, Wright CV. Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature.* 1998; 395(6698):185–189. [PubMed: 9744278]
142. Hong CC, Yu PB. Applications of small molecule BMP inhibitors in physiology and disease. *Cytokine Growth Factor Rev.* 2009; 20(5–6):409–418. [PubMed: 19914855]
143. Hao J, Ho JN, Lewis JA, Karim KA, Daniels RN, Gentry PR, Hopkins CR, Lindsley CW, Hong CC. *In vivo* structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. *ACS Chem Biol.* 2010; 5:245–253. [PubMed: 20020776]
144. Engers DW, Frist AY, Lindsley CW, Hong CC, Hopkins CR. Synthesis and structure-activity relationships of a novel and selective bone morphogenetic protein receptor (BMP) inhibitor derived from the pyrazolo[1,5-a]pyrimidine scaffold of dorsomorphin: the discovery of ML347 as an ALK2 versus ALK3 selective MLPCN probe. *Bioorg Med Chem Lett.* 2013; 23:3248–3252. [PubMed: 23639540]
145. Sanvitale CE, Kerr G, Chaikuad A, Ramel MC, Mohedas AH, Reichert S, Wang Y, Triffitt JT, Cuny GD, Yu PB, et al. A new class of small molecule inhibitor of BMP signaling. *PLoS One.* 2013; 8:e62721. [PubMed: 23646137]
146. Mohedas AH, Wang Y, Sanvitale CE, Canning P, Choi S, Xing X, Bullock AN, Cuny GD, Yu PB. Structure-activity relationship of 3,5-diaryl-2-aminopyridine ALK2 inhibitors reveals unaltered binding affinity for fibrodysplasia ossificans progressiva causing mutants. *J Med Chem.* 2014; 57:7900–7915. [PubMed: 25101911]

147. Fotinos A, Nagarajan N, Martins AS, Fritz DT, Garsetti D, Lee AT, Hong CC, Rogers MB. Bone morphogenetic protein-focused strategies to induce cytotoxicity in lung cancer cells. *Anticancer Res.* 2014; 34:2095–20104. [PubMed: 24778011]
148. Langenfeld E, Deen M, Zachariah E, Langenfeld J. Small molecule antagonist of the bone morphogenetic protein type I receptors suppresses growth and expression of Id1 and Id3 in lung cancer cells expressing Oct4 or nestin. *Mol Cancer.* 2013; 12:129. [PubMed: 24160469]
149. Hao J, Lee R, Chang A, Fan J, Labib C, Parsa C, Orlando R, Andresen B, Huang Y. DMH1, a small molecule inhibitor of BMP type I receptors, suppresses growth and invasion of lung cancer. *PLoS One.* 2014; 9:e90748. [PubMed: 24603907]
150. Ali JL, Lagasse BJ, Minuk AJ, Love AJ, Moraya AI, Lam L, Arthur G, Gibson SB, Morrison LC, Werbowetski-Ogilvie TE, et al. Differential cellular responses induced by dorsomorphin and LDN-193189 in chemotherapy-sensitive and chemotherapy-resistant human epithelial ovarian cancer cells. *Int J Cancer.* 2015; 136:E455–E469. [PubMed: 25227893]
151. Hover LD, Young CD, Bhola NE, Wilson AJ, Khabele D, Hong CC, Moses HL, Owens P. Small molecule inhibitor of the bone morphogenetic protein pathway DMH1 reduces ovarian cancer cell growth. *Cancer Lett.* 2015; 368:79–87. [PubMed: 26235139]
152. Garulli C, Kalogris C, Pietrella L, Bartolacci C, Andreani C, Falconi M, Marchini C, Amici A. Dorsomorphin reverses the mesenchymal phenotype of breast cancer initiating cells by inhibition of bone morphogenetic protein signaling. *Cell Signal.* 2014; 26:352–362. [PubMed: 24280125]
153. Balboni AL, Hutchinson JA, DeCastro AJ, Cherukuri P, Liby K, Sporn MB, Schwartz GN, Wells WA, Sempere LF, Yu PB, DiRenzo J. DeltaNp63alpha-mediated activation of bone morphogenetic protein signaling governs stem cell activity and plasticity in normal and malignant mammary epithelial cells. *Cancer Res.* 2013; 73:1020–1030. [PubMed: 23243027]
154. Owens P, Pickup MW, Novitskiy SV, Giltane JM, Gorska AE, Hopkins CR, Hong CC, Moses HL. Inhibition of BMP signaling suppresses metastasis in mammary cancer. *Oncogene.* 2015; 34:2437–2449. [PubMed: 24998846]
155. the St. Jude Children's Research Hospital-Washington University Pediatric Cancer Genome, P. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet.* 2014; 46:444–450. [PubMed: 24705251]
156. Hover LD, Young CD, Bhola NE, Wilson AJ, Khabele D, Hong CC, Moses HL, Owens P. Small molecule inhibitor of the bone morphogenetic protein pathway DMH1 reduces ovarian cancer cell growth. *Cancer Lett.* 2015; 368:79–87. [PubMed: 26235139]
157. Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Boussein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, et al. BMP type I receptor inhibition reduces heterotopic [corrected] ossification. *Nat Med.* 2008; 14:1363–1369. [PubMed: 19029982]
158. Hao J, Williams CH, Webb ME, Hong CC. Large scale zebrafish-based *in vivo* small molecule screen. *J Vis Exp.* 2010; 46:pii:2243.
159. Mohedas AH, Xing X, Armstrong KA, Bullock AN, Cuny GD, Yu PB. Development of an ALK2-biased BMP type I receptor kinase inhibitor. *ACS Chem Biol.* 2013; 8:1291–1302. [PubMed: 23547776]

Figure 1. Schematic representation of the canonical BMP signalling cascade

Binding of the BMP ligand dimer to its type I and type II receptors forms the BMP transmembrane signalling complex. Within this complex, the type II receptor serine–threonine kinase phosphorylates and activates the type I receptor. The canonical signalling occurs when type I receptor serine–threonine kinase phosphorylates SMAD1/5/8, leading to the formation of the SMAD1/5/8–SMAD4 complex. The SMAD1/5/8–SMAD4 complex then translocates to the nucleus, where it activates the expression of BMP target genes such as *Id1*, *-2* and *-3*. BMP signalling is regulated at multiple steps. Extracellular antagonists can complex with BMP ligand to prevent binding to its receptor. Smurf1/2 and SMAD6/7 can prevent phosphorylation of SMAD1/5/8 and binding to its co-factor SMAD4. Small molecule BMP inhibitors, such as dorsomorphin (DM) and DMH1, are competitive kinase inhibitors of BMP type I receptor. Inside the green insert are the non-canonical (SMAD-independent) signalling pathways, p38, LIMK, Rho and ROCK. Not shown are additional BMP pathway modulators such as co-receptors, which can modulate ligand-receptor binding affinity, and pseudoreceptors, which block receptor activation.

	Ligand	Type I Rec.	Type II Rec.	R-Smad
BMPs/GDFs	BMP2/4	ALK3, 6	BMPR2 ActR-IIA ActR-IIB	Smad1/5/8
	BMP5/6/7/8	ALK2, 3, 6		
	GDF5/6/7	ALK3, 6		
	BMP9/10	ALK1		
	GDF1/3	ALK4	ActR-IIA ActR-IIB	Smad2/3
	BMP3/GDF10	ALK4		
	GDF8/11	ALK4, 5		
	BMP15	ALK6	BMPR2	Smad1/5/8
	GDF9	ALK4, 5	BMPR2	Smad2/3
	TGF β s	ALK5	TGFR2	Smad2/3
		ALK1 + Endoglin		Smad1/5/8
activins	Activin A	ALK4	ActR-IIA ActR-IIB	Smad2/3
	Activin B	ALK4, 7		
	Nodal	ALK4, 7	ActR-IIB	Smad2/3
other	AMH	ALK2, 3, 6	AMHRII	Smad1/5/8
	GDF15	Unknown	Unknown	Unknown

Figure 2. Partial list of TGF- β /BMP ligands, and respective receptor and R-SMAD activation
TGF- β superfamily ligands are arranged according to phylogenetic analysis [5]. In some cases, involvement of co-receptors (Endoglin) are represented. The yellow box represents ligand–receptor interactions that trigger SMAD1/5/8 signalling cascade, and the blue box represents ligand–receptor interactions that trigger SMAD2/3 signalling cascade. The hatched box represents ligand–receptor interactions that trigger either cascade depending on the presence of a co-receptor Endoglin. This is a simplified representation of very complex sets of molecular interactions and not meant to be comprehensive. Modified from [5]: Mueller, T.D. and Nickel, J. (2012) Promiscuity and specificity in BMP receptor activation. *FEBS Lett.* **586**, 1846–1859; and [8]: Yadin, D., Knaus, P. and Mueller, T.D. (2016) Structural insights into BMP receptors: specificity, activation and inhibition. *Cytokine Growth Factor Rev.* **27**, 13–34.

Figure 3.
Chemical structure of published small molecule BMP inhibitors

Table 1
Nomenclature of TGF β /BMP type I and type II receptors

Although each receptor is known by multiple names, accepted symbol for the gene encoding each BMP receptor is italicized in the right column. Protein symbol for each receptor used in the present review is bold faced.

	Alternative names
Type I receptors	
Activin receptor-like kinase 1	<i>ACVRL1</i> , ALK1
Activin A receptor, type I	<i>ACVR1</i> , ALK2 , ACTRI, ACVR1A, SKR1, TSR1, ACVRLK2
Bone morphogenetic protein receptor, type IA	<i>BMPRIA</i> , ALK3 , CD292, SKR5, ACVRLK3
Bone morphogenetic protein receptor, type IB	<i>BMPRIB</i> , ALK6
Activin receptor, type IB	<i>ACVR1B</i> , ALK4 , ACTRIB; ACVRLK1; ALK4; SKR2
Transforming growth factor-beta receptor 1	<i>TGFBRI</i> , ALK5 , ACVRLK4
Activin A receptor, type IC	<i>ACVR1C</i> , ALK7 , ACVRLK7
BMP type II receptors	
Bone morphogenetic protein receptor, type II	<i>BMPR2</i> , BMPR2 , BMPR-II, BMR2, PPH1, BMPR3, BRK-3, POVD1, T-ALK
Activin A receptor, type IIA	<i>ACVR2A</i> , ActR-IIA , ACTRII, ACVR2
Activin A receptor, type IIB	<i>ACVR2B</i> , ActR-IIB , ACTRIIB, HTX4
Transforming growth factor-beta receptor II	<i>TGFRB2</i> , TGFR2 , TGF- β -RII

Table 2
Partial list of studies elucidating the role of BMP signalling in cancer

Studies are listed based on different levels of evidence from human tumour data to *in vitro* cellular and *in vivo* preclinical data.

	Studies
Acute megakaryoblastic leukaemia (AMKL, non-Down syndrome)	<p>Human somatic mutation: CBFA2T3-GLIS2 fusion protein identified in non-DS-AMKL patients. Overexpression of BMP2/4 observed in non-DS-AMKL patients expressing fusion gene (Gruber et al. [33]).</p> <p><i>In vitro</i>: Increased Id1 expression and ability of cells to self-renew in murine hematopoietic cells transfected with chimeric transcript; effect inhibited by DM.</p> <p><i>In vivo</i>: BMP gain-of-function phenotypes in <i>Drosophila</i> expressing CBFA2T3-GLIS2, e.g. shortened, blistered wings and ectopic wing veination.</p>
Diffuse intrinsic pontine glioma (DIPG)	<p>Human somatic mutations: 7 gain-of-function somatic mutations in <i>ACVR1</i> (ALK2) were identified in patients with DIPG (R206H, Q207E, R258G, G328V, G328W, G328E and G356D) (St Jude Children's Research Hospital-Washington University Pediatric Cancer Genome Project [155], Buczkowicz et al. [50], Taylor et al. [51], Fontebasso et al. [52]). 5 of the 7 mutations identical with germline mutations in fibrodysplasia ossificans progressiva (FOP).</p> <p><i>In vitro</i>: Increased levels of pSMAD1/5 in human DIPG samples with R206H and G328E mutations in <i>ACVR1</i> gene. Increased expression of Id1 and Id2 in G328V mutant human astrocytes. Increased levels of pSMAD 1/5 in G328E, G328V, R258G, G356D and R206H mutant mouse astrocytes; SMAD phosphorylation inhibited by LDN-193189.</p> <p><i>In vivo</i>: Disruption of dorsoventral patterning and loss of head and dorsal structures in zebrafish expressing G328V, G356D, R206H, G328W, G328E, R258G mutations; partial rescue of ventralization phenotype with LDN-193189.</p>
High-grade glioma (HGG)	<p>BMP signalling is active in certain high-grade gliomas (HGG) obtained from adult patients (Hover [55]). Deletion of <i>BMPR1A</i> (ALK3) in oncogenic mouse astrocytes resulted in decreased proliferation, and invasion, migration. In addition, in a mouse model of HGG involving orthotopic transplantation of transformed astrocytes, the genetic knockout of <i>BMPR1A</i> (ALK3) showed a dramatic increase in survival. <i>In vitro</i> effects of BMPR1A ablation was recapitulated with DMH1 [55].</p>
Lung Cancer	<p>Alterations in gene expression: 98 % of human NSCLC aberrantly overexpress BMP2 (Langenfeld [57]). Decreased 1-year survival rate in patients with elevated serum BMP2 (Choi et al. [59]). High BMP2 expression associated with lymph node metastasis and advanced tumour stage (Chu et al. [60]).</p> <p><i>In vitro</i>: In A549 lung adenocarcinoma cells with increased expression of BMP2, LDN-193189 reduced cell growth and promoted cell death as measured by increased LDH release (Fontinos et al. [147]). BMP2 dose-dependent stimulation of cell migration and invasion in A549 and H7249 lung cancer cell lines; effect completely inhibited by Noggin (Langenfeld et al. [62]). Growth inhibition and cell death of H1229 and A549 lung cancer cell lines as well as decreased phosphorylation of SMAD1/5/8 and Id1-3 expression using small molecule BMP antagonists (Langenfeld et al. [64]). DMH1 reduced cell invasion and migration of A549 cells (Hao et al. [149]).</p> <p><i>In vivo</i>: BMP2 enhanced tumour growth and angiogenesis in murine model of lung cancer (Langenfeld et al. [63]). DMH1 significantly reduced tumour growth in mouse model of NSCLC (Hao et al. [149]).</p>
Colorectal cancer	<p>Alterations in gene expression. Germline mutations in BMPR1A and SMAD4 in roughly 50 % of individuals with juvenile polyposis (Jass et al. [16], van Hattem et al. [17], Calva-Cequeria et al. [18]). Increased BMP7 expression associated with increased tumour aggression and worse clinical prognosis (Motoyama et al. [28]) in patients with colorectal cancer.</p> <p><i>In vitro</i>: Enhanced migration and invasion of HCT116 cells with overexpression of BMP4 (Deng et al. [24]).</p>
Pancreatic cancer	<p>Alterations in gene expression: Increased expression of BMP2/4/7 in human pancreatic cancer specimens.</p> <p><i>In vitro</i>: BMP-induced EMT leading to increased invasiveness and up-regulation of matrix metalloproteinase-2 (MMP-2) in Panc-1 cells (Gordon et al. [66]).</p>
Ovarian cancer	<p>Alterations in gene expression: Overexpression of BMP2/4 and Id1/3 in primary human ovarian cancer cell lines (Shepherd et al. [75,76]). Amplification of BMP ligands in human ovarian cancers, correlation of BMP receptor expression with poor prognosis, and active BMP signalling in human ovarian tissue (Hover et al. [156]).</p> <p><i>In vitro</i>: DM or LDN-193189 decreased viability of EOC216 ovarian cancer cell line (Ali et al. [150]). DMH1 reduced ovarian tumour sphere growth and enhanced sensitivity of ovarian cancer cells to Cisplatin treatment (Hover et al. [156]).</p> <p><i>In vivo</i>: In mouse model of serous ovarian cancer, use of recombinant BMP2 promoted significant tumour growth, an effect that was inhibited by DM (Peng et al. [80]). DM also significantly increased median survival time in a mouse xenograft model of epithelial ovarian cancer (Ali et al. [150]).</p>

	Studies
Osteosarcoma	<p>Alterations in gene expression: Overexpression of BMP2/4 and BMP6 in high-grade osteosarcoma with malignant fibre histiosarcoma (MFH)-type pattern and chondroblastic osteosarcoma respectively (Yoshikawa et al. [83], Sulzbacher et al. [84]). Co-expression of BMP-2/4 and BMP2 associated with reduced 5-year survival rate (Yoshikawa et al. [87]).</p> <p><i>In vitro</i>: BMP2 enhanced heptotactic migration of murine osteosarcoma Dunn cells to several extracellular matrix ECM components, including fibronectin, type I collagen, and laminin-1, and promoted cell migration, cell proliferation, and focal adhesion formation via modulation of fibronectin-integrin-β1 signalling. Noggin abrogated the stimulatory effects of BMP2 (Sotobori [90]).</p>
Prostate cancer	<p>Alterations in gene expression: BMP-6 expression detected in over 50 % of patients with metastatic prostate cancer; absent from non-metastatic or benign-prostate samples (Bentley et al. [92]). Higher-grade human prostate tumours (Gleason score of 6 or more) have increased BMP6 immunostaining than lower-grade human prostate tumours (Gleason score of 4 or less) (Barnes et al. [95]). BMP6 mRNA detected exclusively in malignant epithelial cells in metastatic prostate specimens, but undetectable in benign prostate samples (Hamdy et al. [94]). Positive correlation between epithelial staining for Id-1 and BMP-6 in prostate cancer samples (Darby et al. [96]).</p> <p><i>In vitro/in vivo</i>: BMP6 enhanced rate of cell migration and invasion in PC3M and DU145 cell lines (Darby et al. [96]). Anti-BMP6 attenuated LuCaP 23.1-induced osteoblastic activity and reduced interosseous tumour size in mouse model of prostate cancer (Dai et al. [97]).</p>
Breast cancer	<p>Alterations in gene expression: Elevated expression of BMPR1A and BMPR2 associated with poor relapse-free survival time (Owens et al. [115]).</p> <p>Overexpressing BMP7 primary tumours associated with accelerated bony metastasis (Alarmo et al. [118]).</p> <p><i>In vitro</i>: BMP2 enhanced cell migration in MCF-7 cells and increased expression of BCSG1, breast-cancer-specific gene (Clement et al. [106]).</p> <p><i>In vivo</i>: In mouse model of breast cancer, BMP2 formed tumours with enhanced vasculature and increased Id1 and p38 MAPK activation (Clement et al. [106], Rada et al. [107]). DMH1 treated mice with breast cancer had reduced primary tumour burden and lung metastasis as well as decreased lymphatic vessel growth and expression of EMT markers e.g. Snail, Twist (Owens et al. [115]). Deletion of the BMP receptor BMPR1A impairs mammary tumour formation and metastasis in a mouse genetic model expressing MMTV.PyMT oncogene (Pickup et al. [117]).</p>
Melanoma	<p>Alterations in gene expression: Enhanced immunostaining of BMP4/7 in primary and metastatic melanoma specimens (Rothhammer et al. [123]).</p> <p><i>In vitro</i>: Significant inhibition of cellular invasion in stably transfected chordin or BMP4 antisense cell lines.</p> <p><i>In vivo</i>: BMP2 enhanced melanoma cell migration in chick embryos transplanted with B16-F1 cells; melanoma cell migration inhibited by Noggin (Busch et al [124])</p>
Head and neck squamous cell cancer	<p>Alterations in gene expression: 98 % of HNSCC express baseline BMP-2 expression. High levels of BMP2 expression associated with increased incidence of local failure rate (Sand et al. [132]). Overexpression of BMP2 associated with increased lymph node metastasis and extracapsular spread of metastatic lymph nodes (Zhou et al. [134]).</p> <p><i>In vitro</i>: BMP4 promoted cell invasion and migration in Tu686 and Tu212 cell lines (Xu et al. [135]).</p> <p><i>In vivo</i>: Mouse model of oral squamous cell carcinoma treated with rhBMP2 had reduced survival and rapid local tumour growth as well as poorly differentiated tumour morphology (Kokorina et al. [133]).</p>

Table 3
Inhibition of selected kinases *in vitro* by small molecule BMP inhibitors

Results of *in vitro* kinases assays for ALK1-6, BMPR2, TGF- β type II receptor (TGFR2) and VEGF receptor (KDR) are shown as IC50 (concentration at 50 % inhibition). IC50s for *in vitro* kinase assay are not identical with the published IC50s for cell based studies described in the text. *Results obtained by CCH, which may vary from published reports based on experimental conditions. n.d.: not determine. NO: no inhibition at highest concentration tested (>30–100 μ M).

IC50 (nM)	Dorsomorphin (DM)* [140]	LDN-193189 [145, 157]	DMH1* [158]	DMH2* [158]	DMH3* [158]	ML347* [144]	K02288 [145]	LDN-212854* [159]	LDN-214117 [146]
ALK1	100	1	25	10	5	45	2	55	27
ALK2	60	1	100	40	25	30	1	15	27
ALK3	95	5	<5	<5	<5	>10000	34	120	1171
ALK6	230	17	48	<5	10	>9000	6	220	3000
ALK4	>20000	100	>9000	1400	640	NO	302	>9000	n.d.
ALK5	>17000	350	NO	2400	2000	NO	321	>11000	n.d.
BMPR2	70	n.d.	NO	>3800	2200	NO	n.d.	n.d.	n.d.
TGFR2	100	n.d.	NO	86	246	NO	n.d.	n.d.	n.d.
KDR	20	556	NO	2400	2000	>19000	>10000	2770	n.d.