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Targeting Tumor Vasculature by Inhibiting Activin Receptor-like Kinase (ALK)1 Function

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Abstract

Angiogenesis is a hallmark of cancer and is now a validated therapeutic target in the clinical setting. Despite the initial success, anti-angiogenic compounds impinging on the vascular endothelial growth factor (VEGF) pathway display limited survival benefits in patients and resistance often develops due to activation of alternative pathways. Thus, finding and validating new targets is highly warranted. Activin receptor-like kinase (ALK)1 is a transforming growth factor beta (TGF-β) type I receptor predominantly expressed in actively proliferating endothelial cells (ECs). ALK1 has been shown to play a pivotal role in regulating angiogenesis by binding to bone morphogenetic protein (BMP)9 and 10. Two main pharmacological inhibitors, an ALK1-Fc fusion protein (Dalantercept/ACE-041) and a fully human antibody against the extracellular domain of ALK1 (PF-03446962) are currently under clinical development. Herein, we briefly recapitulate the role of ALK1 in blood vessel formation and the current status of the preclinical and clinical studies on inhibition of ALK1 signaling as an anti-angiogenic strategy. Future directions in terms of new combination regimens will also be presented.

Key Words: angiogenesis, tumor vasculature, activin receptor-like kinase 1 (ALK1), bone morphogenetic protein

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; AE, adverse effects; ALK1, activin receptor-like kinase 1; AVMs, arteriovenous malformations; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; BRE, BMP responsive element; CAM, chorioallantoic membrane; ECs, endothelial cells; GI, gastrointestinal; HHT, hereditary hemorrhagic telangiectasia; HUVEC, human umbilical vein endothelial cells; MTD, maximum tolerated dose; MMTV-PyMT, mouse mammary tumor virus - polyoma middle T antigen; RIP1-TAg2, rat insulin promoter – SV40 large

T antigen; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor.

ALK1 signaling in angiogenesis

Angiogenesis is a tightly regulated process where new blood vessels develop from a pre-existing capillary network [1,2]. The vascular tree is formed during embryonic and early postnatal development, and reaches a quiescent state in adulthood, with the exceptions of wound healing and the female reproductive cycle. However, pathological activation of angiogenesis occurs in several human diseases, such as rheumatoid arthritis, diabetic retinopathy, cardiovascular disorders, tumor growth and its progression [3]. In solid malignancies, the induction of an "angiogenic switch" acts as an essential requirement for the outgrowth of tumors beyond a certain size, supplying oxygen and nutrients to the cancer cells, as well as removing waste products and providing an escape route for metastatic dissemination [3,4].

Initially, the action of transforming growth factor beta (TGF-β) in regulating angiogenesis was largely underappreciated, as in the late eighties and nineties studies on TGF-β mainly focused on its regulation of epithelial and mesenchymal growth. Extensive evidence in human and mouse has thereafter revealed a fundamental role for the TGF-β signaling pathway in angiogenesis and vascular remodelling [5]. TGF-β is part of a larger family of structurally and functionally related extracellular cytokines, which also includes activins, inhibins, growth and differentiation factors (GDFs) and bone morphogenetic proteins (BMPs). In addition, many family members different from TGF-β are emerging as powerful vascular morphogens [6,7]. All TGF-β family members signal *via* specific complexes of type I serine/threonine kinases (known as activin receptor-like kinases (ALKs)) and type II receptors, further inducing different sets of

intracellular Smad transcriptional regulators [8]. Genetic studies in mouse and human have shown that deletion of components of the TGF-β family, such as the TGF-β type II receptor (TβRII), type I receptors ALK1 and ALK5, and accessory co-receptor endoglin, downstream effectors Smad1 and Smad8, leads to embryonic lethality due to vascular abnormalities [9]. Among them, ALK1 has been shown to be a crucial player during vascular development. ALK1 is predominantly expressed on proliferating vascular endothelial cells (ECs) [10] and was initially found to weakly interact with TGF-β [11]. Only years later, BMP9 and BMP10 were described as the high affinity ligands for the ALK1 receptor [12-14]. Activation of ALK1 triggers intracellular signaling *via* phosphorylation of receptor-regulated Smad1/5/8 (Figure 1). Mice lacking the gene encoding ALK1 (*Acvrl1*) die at embryonic day 11.5 due to severe vascular abnormalities [11,15,16], whereas *Acvrl1* heterozygous mice develop vascular lesions such as arteriovenous malformations (AVMs) in the spleen, lung and liver. This altered phenotype was also observed upon conditional deletion of *Alk1* in ECs [17]. Furthermore, patients carrying a genetic mutation in *ACVRL1* develop hereditary hemorrhagic telangiectasia 2 (HHT2), a disease characterized by abnormal vessel formation [18] and similar to HHT1, which is instead caused by a mutation in endoglin (*ENG)* [19]. Together, these studies demonstrate that ALK1 is required for angiogenesis. However, the exact function of ALK1 in angiogenesis remains controversial. One study proposed that ALK1 signaling inhibits the proliferation, migration and adhesion of ECs and therefore, it could possibly be implicated in the maturation phase of angiogenesis [20]. In an opposite way, another study showed that ALK1 pathway promotes proliferation and migration of ECs [21]. The intricate nature of the response induced upon ligand binding to ALK1, and possible recruitment of lower affinity receptors at high ligand concentrations, remains a challenge in the field, thus emphasizing the context- and

dose-dependency of such interactions. Despite the wide range of studies performed [14,22-24], no conclusive assumption can be made. For example, *in vitro* stimulation with either BMP9 or TGF-β alone led to inhibition of tube formation and proliferation of endothelial cells. On the contrary, a stimulatory effect was observed when BMP9 and TGF-β were supplemented in combination. Similar results were obtained in a matrigel plug assay of VEGF-A/bFGF-induced angiogenesis *in vivo* [24] .

In addition, ALK1 engages in crosstalks with other pathways, such as VEGF and Notch, as well as with other ALK-regulated pathways, highlighting the involvement and multifaceted role of ALK1 at different stages of angiogenesis [25,26].

A special mention should also be made to the ALK1 co-receptor endoglin, as it is a key player during angiogenesis. Endoglin modulates TGF-β signaling through ALK5 or ALK1, favouring ALK1 activation while inhibiting TGF-β/ALK5 pathway [27] (Figure 1). Indeed, endoglin also binds to other ligands, apart from BMP9 and BMP10, such as TGF-β_1 and TGF-β_3, but then in addition requires the presence of TβRII [28].

Inhibition of ALK1 function as anti-angiogenic therapy in cancer

Angiogenesis is considered one of the hallmarks of cancer and therefore its inhibition is an established therapeutic strategy in cancer treatment [4]. The main goal of anti-angiogenic regimens is to abrogate the formation of new blood vessels within the tumor, depriving cancer cells from oxygen and nutrients and consequently inhibiting growth and progression. Moreover, tumor angiogenesis gives rise to an aberrant vascular network, contributing to the generation of interstitial hypertension, acidosis and hypoxia. This peculiar abnormal microenvironment jeopardizes the efficacy and proper delivery of therapeutics to solid tumors. Therefore, additional benefits might be achieved by

combining anti-angiogenic agents with other cytotoxic drugs.

Molecules targeting several pathways, with VEGF being the most common, are currently being examined aiming to reach clinical settings. However, despite the initial success and improvement in response rate in several cancers, the overall survival is only modestly improved and resistance to therapy often develops due to activation of alternative pathways [29]. Therefore, novel targets are urgently needed. In this sense, following VEGF inhibition, upregulation of endoglin has been reported. However, no direct link between BMP9/ALK1 levels and acquired resistance to anti-VEGF therapy has been proven [30].

The predominant expression of ALK1 on ECs and its upregulation in neoangiogenic circumstances made it an attractive target for anti-angiogenic therapies [31,32]. Different biological compounds have been designed to interfere with ALK1 signaling and exploited for therapeutic intervention against tumor angiogenesis (Figure 2). Among them, small molecule inhibitors of the BMP type I receptors, such as LDN-193189, have been shown to inhibit ALK1 signaling by selectively targeting its kinase activity (Figure 2) [33-35]. However, specificity of such drugs and crosstalk with other pathways make it difficult to predict the final outcome of these inhibitions. Almost a decade ago, two pharmaceutical companies started independent phase I clinical trials with ALK1-targeting agents in solid tumors using two different strategies: a fully human antibody against the extracellular domain of ALK1 (PF-03446962) developed by Pfizer [36] and an ALK1-Fc fusion protein (Dalantercept/ACE-041) developed by Acceleron Pharma [37]. Theoretically, these two approaches aiming to block ALK1 signaling could lead to different consequences. The anti-ALK1 antibody could inhibit the binding and downstream signaling of all ligands that activate ALK1 (not only BMP9 and BMP10, but also TGF-β and others) [38], leaving their capability to bind to related receptors, such

as ALK5 for TGF-β or ALK2 for BMP9, on ECs or other cell types unaltered (Figure 2). The ALK1-Fc chimeric protein could sequester the high affinity ligands that bind directly to ALK1 (BMP9 and BMP10), but at the same time could preserve the activation of ALK1 through other ligands, such as TGF-β, if TβRII is available [39].

PF-03446962 is a fully human monoclonal antibody that targets and neutralizes the human ALK1 receptor. *In vitro* studies showed that PF-03446962 prevented binding of BMP9 to ECs [38]. PF-03446962 binds with high affinity to residues 42-56 in the human ALK1 extracellular domain but does not bind other closely related ALKs, such as ALK2, ALK3, ALK4, ALK5 or ALK7, reducing potential off-target effects. *In vitro* assays showed that PF-03446962 inhibited BMP9-induced Smad phosphorylation and BMP responsive element (BRE)-luciferase transcriptional reporter activity. In addition, the monoclonal antibody could block serum-induced Smad1 phosphorylation, migration and tube formation in HUVECs, as well as endothelial sprouting [38,40].

Dalantercept is a chimeric protein consisting of human ALK1 extracellular domain (residues 1-99) fused to the Fc fragment of human immunoglobulin G1 (IgG1) [41]. Dalantercept (and its mouse counterpart RAP-041) acts as a ligand trap binding circulating ligands, blocking the activation of endogenous ALK1 [42]. Dalantercept binds with high affinity to BMP9 and BMP10, but not to any other ligand of the TGF-β family [42]. *In vitro* experiments showed that ALK1-Fc could block BMP9- and BMP10-induced Smad1 phosphorylation and downstream signaling (e.g. *Id1* gene expression). In a functional assay, Dalantercept blocked human umbilical vein ECs (HUVEC) cord formation and endothelial sprouting. Moreover, Dalantercept inhibited FGF-induced neovascularization and VEGF-induced vessel formation in a chick chorioallantoic membrane (CAM) assay [42].

Targeting of ALK1 *in vivo*

Anti-angiogenic therapy based on ALK1 inhibition involves direct modification of the tumor vasculature, with consequential changes in the primary tumor growth and the close-by microenvironment, as well as potential impact on metastasis formation.

The initial characterization of the *in vivo* properties of RAP-041 (and its human counterpart Dalantercept) was made in RIP1-TAg2 model of pancreatic neuroendocrine tumorigenesis, that faithfully recapitulates the main features of the human disease [43]. Administration of the ALK1 ligand trap resulted in an impaired tumor growth already after two weeks [24], whereas a longer four-week treatment also decreased the number of hepatic micrometastases compared with the control cohort [44]. Lower transcript levels for genes downstream of ALK1 stimulation, such as Id1 and Id3, demonstrated on-target effects of RAP-041 activity [24]. Interestingly, inhibition of ALK1 also affected TGF-β/ALK5 signaling, as evidenced by the reduced PAI-1, Fibronectin and PDGFB gene expression, further demonstrating interdependence of the pathways [24]. Similar results were obtained in the transgenic spontaneous mouse mammary tumor virus (MMTV)-polyoma middle T antigen (PyMT) and the syngeneic transplantable EO771 models of breast cancer [44]. Likewise, tumor growth was delayed in human MCF7 and MDA-MB-231 xenografts when using either Dalantercept or PF-03446962 [40,42,44]. However, in a second study of poorly/non-metastatic melanoma, breast, head and neck cancer, no differences in the tumor burden could be observed at the experimental endpoint when RAP-041 was used as a monotherapy [45].

The changes in the vascular network and the subsequent alterations in the local microenvironment have been consistently reported in numerous mouse models of cancer. Neoadjuvant therapy with ALK1-targeting agents impinged directly on the vasculature and in-depth characterization revealed reduced vessel density and increased

pericyte coverage, usually associated with decreased sprouting and leakiness [24,40,44]. Nonetheless, contradicting data emerged when assessing the functionality of the tumor-associated vasculature following administration of either RAP-041 or PF-03446962. On the one hand, RAP-041 led to impaired vascular perfusion, a finding that was corroborated in experimental tumors exposed to PF-03446962, as well as in phase I clinical trials [24,36,37,40,44]. Hu-Lowe and colleagues showed that flow rates were affected only in large, functional blood vessels, while smaller ones were left unaltered [40]. On the other hand, work by Hawinkels and others described a slight increase in the perfusion and this may explain the improved effects when delivering the ALK1 ligand trap together with clinically approved cytotoxic compounds [45].

Combined use of chemotherapeutic agents (*e.g.* Cisplatin, Docetaxel and Doxorubicin) and RAP-041 enhanced the tumor growth inhibition in several murine models of solid cancer [44,45]. In experimental breast carcinomas, the blunted vessel density caused by RAP-041 was further diminished in the combined therapy group, accompanied by an equally significant reduction of the metastatic count in the lungs. Of note, co-administration of RAP-041 and other anti-angiogenic drugs, *i.e.* the VEGFR2 neutralizing-antibody DC101, only produced negligible benefits [44]. Interestingly, tumors previously resistant to VEGF inhibitors showed a significant decrease in the tumor burden and associated vasculature when exposed to PF-03446962, through a mechanism that might involve the disruption of the vessel normalization phenotype typical of bevacizumab [40]. Another rationale is that the two pathways control sequential steps in angiogenesis, VEGFR stimulation being responsible for its proliferation phase and ALK1 activation for the maturation phase of the process.

One of the major compensation mechanisms following anti-VEGF treatment is the induction of intratumoral hypoxia to stimulate the formation of new vessels for nutrients

and oxygenation supply. As a consequence, in different pre-clinical models, tumors quickly acquire resistance, leading to more locally invasive tumors that also display an augmented metastatic seeding potential. Notably, RAP-041 did not provoke a widespread hypoxic response, possibly one of the causes of the reduced metastatic burden observed in the MMTV-PyMT breast cancer model [44]. No overt differences were detected in the tumor microenvironment upon ALK1 inhibition with the ligand trap, as macrophage infiltration and tumor cell proliferation were not significantly altered [24,44]. Increased apoptosis (quantified as DNA fragmentation by TUNEL) was observed *in vivo* in the context of the RIP1-Tag2 tumorigenesis [24], but this is likely a secondary effect of the compound, as *in vitro* experiments did not show any direct induction of cell death after delivery of RAP-041.

Furthermore, lymphangiogenesis is tightly regulated by the BMP9/ALK1 axis and administration of PF-03446962 resulted in the reduction of lymphatic vessel density. Surprisingly, stimulation of ALK1 signaling by BMP9 elicited the same net outcome in a model of breast cancer [46], highlighting the potential compensation of other ligands and their interplay within this pathway. The intrinsic differences in the mode of action of RAP-041 and PF-03446962, together with context-specific and concentration-dependent interactions, should be taken into account when considering the biological responses to such compounds (summarized in Table 1). The exact role of BMP9/ALK1 signaling in the tumor-associated lymphatics is currently under investigation, given the relevant clinical implications reported on the first-in-human trials (see next paragraph).

Therapeutic use

The remarkable *in vivo* effects of the two ALK1-targeting agents prompted phase I clinical trials in patients with advanced cancer. The primary end point to determine the

maximum tolerated dose (MTD) was achieved in two independent trials, with already detectable anti-tumor activity in patients (measured as partial response and/or prolonged stable disease) and clear signs of on-target effects, such as decreased tumor blood flow, perfusion and microvascular density detected with magnetic resonance imaging (MRI) and ultrasound [36,37].

Dalantercept and PF-03446962 were generally well-tolerated and had distinct safety profiles from that of anti-VEGF therapy, underlining the diverse expression pattern of the receptors in the vasculature: VEGFR2 is constitutively and ubiquitously expressed, whereas ALK1 is mainly expressed in actively proliferating ECs [10,47]. None of the patients recruited in these studies displayed the most severe adverse effects (AE) reported following bevacizumab treatment, including gastrointestinal (GI) perforation, impaired wound healing, serious bleeding and critical hypertension. The most commonly observed AE upon Dalantercept administration were peripheral edema, fatigue and anemia, while fatigue, nausea and thrombocytopenia (not associated with bleeding) were typical of PF-03446962 infusion [36,37]. Fluid retention was considered the major dose-limiting factor in patients treated with Dalantercept, which may point towards inhibition of the lymphangiogenesis process. Moreover, as a proof principle of ALK1 inhibition, some of the patients enrolled in these trials developed telangiectasia, a condition observed in the HHT1 and HHT2 syndromes as a consequence of endoglin and ALK1 haploinsufficiency, respectively. Telangiectasia was indeed reported in the first-in-human phase I clinical trials with the chimeric anti-endoglin antibody TRC105. Of note, TRC105 showed a safety profile not overlapping with those of bevacizumab and ALK1-targeting agents. The most common adverse effects reported were infusion reaction (given the antibody-dependent cellular cytotoxicity (ADCC) mode of action),

headache and fatigue. Moreover, as endoglin is expressed also by proerythroblasts, some patients experienced clinically reversible anemia [48].

Several phase II trials with either Dalantercept or PF-03446962 as a monotherapy have now been initiated in a diverse range of malignancies, including squamous cell carcinoma of the head and neck, endometrial cancer, ovarian cancer, urothelial cancer, liver cancer and pleural mesothelioma (see Table 2). So far, the only completed (and published) study refers to recurrent or persistent endometrial cancer, where Dalantercept failed to reach the intended primary endpoint due to its insufficient activity [49]. The phase II trial of Dalantercept in head and neck cancer, which was motivated by promising responses of patients with this disease in the phase I trial, has been completed, but is yet to be reported.

High hopes are concentrated over the numerous phase II combination trials in different malignancies: Acceleron Pharma has very recently finished recruiting or is currently recruiting patients to test the efficacy of Dalantercept in combination with sorafenib and axitinib in advanced hepatocellular carcinoma and renal cell carcinoma, respectively [41]. In a similar fashion, Pfizer is actively expanding the cohorts for combined PF-03446962 and regorafenib in colorectal cancer (clinicaltrials.gov) (Table 2).

Conclusions

Targeting of ALK1 using a neutralizing ALK1 antibody (PF-03446962) or ALK1-Fc ligand trap (Dalantracept) has shown beneficial effects on inhibiting tumor angiogenesis, alone or in combination with chemotherapy. Clinical trials are reporting favorable effects with tolerable side effects. Future trials will likely explore more combinatorial treatments of ALK1-blocking agents with other targeted therapies, including newly developed immune checkpoint inhibitors.

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Figure legends

Figure 1

TGF-β and BMP9 signaling in endothelial cells. Schematic representation of TGF-β and BMP9 signaling *via* their serine/threonine type I and type II receptors, co-receptor Endoglin and downstream Smad intracellular proteins. TGF-β binds to TβRII, and can recruit ALK5 or ALK1. Type I receptors are phosphorylated by TβRII. TGF-β/ALK5 induces Smad2 and Smad3 phosphorylation while TGF-β/ALK1 mediates Smad1 and Smad5 phosphorylation. TGF-β/ALK1 also requires ALK5. Endoglin facilitates TGF-β/ALK1signaling and inhibits TGF-β/ALK5 signaling. BMP9 binds to heteromeric complexes of BMPRII and ActRII and ALK1 and ALK5. In particular, ALK1 is a high affinity receptor for BMP9. BMP9/ALK1 (or BMP9/ALK2) induces Smad1 and Smad5 phosphorylation. Activated R-Smads form heteromeric complexes with the common mediator Smad4, and these can accumulate in the nucleus to regulate gene transcriptional responses. ALK5/Smad3 activation promotes CAGA12-luc transcriptional reporter and *plasminogen activator inhibitor-1 (PAI-1)* gene expression, among many others. ALK1/Smad1 or Smad5 activation mediates BRE-luc transcriptional response and stimulates the expression of *Id1* and I*d3* target genes.

Figure 2

Targeting ALK1 signaling. (**a**) PF-03446962 antibody against ALK1 inhibits TGF-β and BMP9/ALK1 signaling by binding to ALK1 and therefore interfering with ligand binding. TGF-β/ALK5 (not shown in this figure) and BMP9/ALK2 signaling are not affected by PF-03446962. (**b**) ALK1-Fc ligand trap (Dalantercept) sequesters BMP9

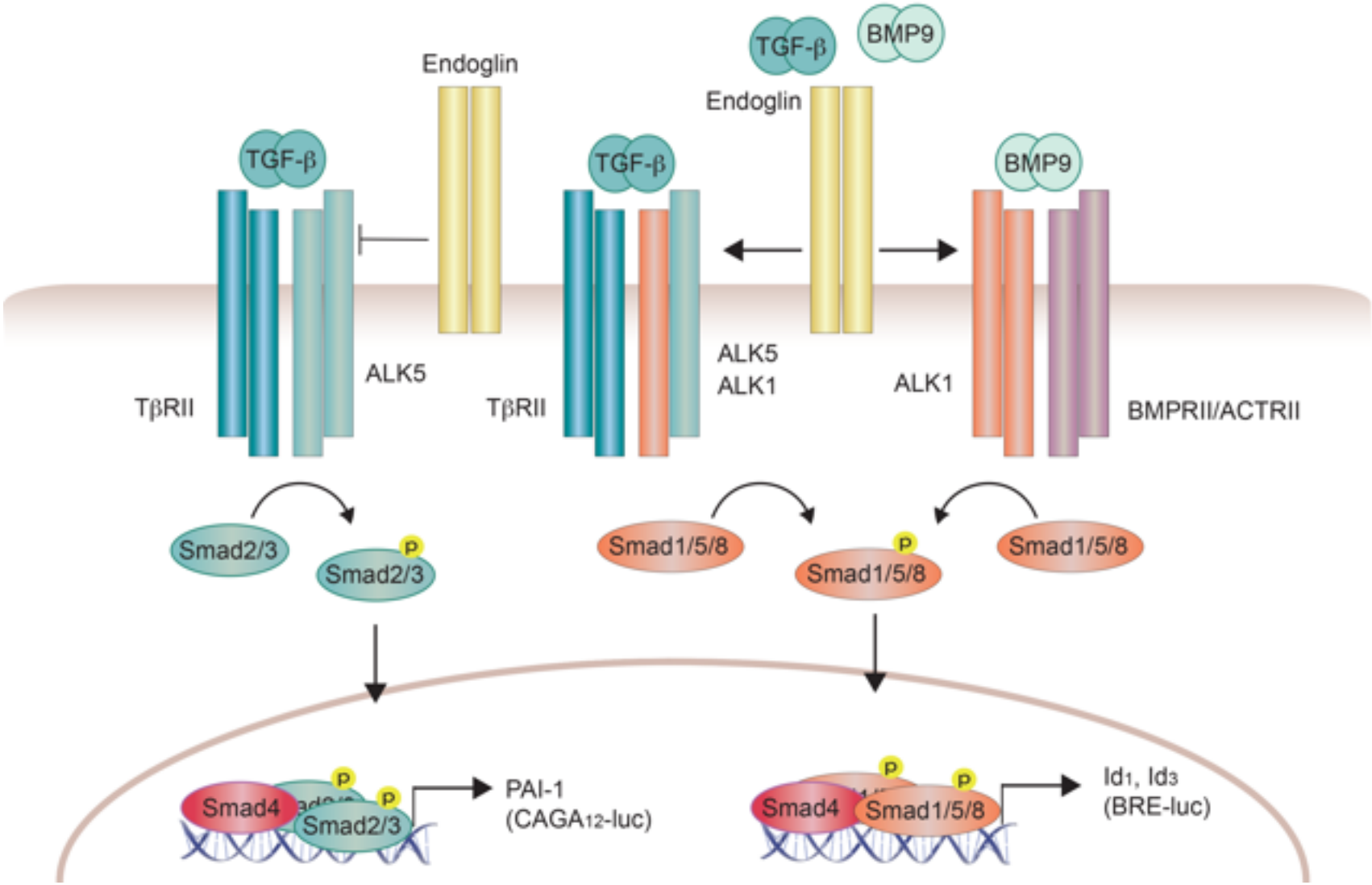
(and BMP10) and prevents them from binding to cell surface receptors. ALK1-Fc does not interfere with signaling of the low affinity ALK1 ligands like TGF-β (**c**) ALK1 kinase inhibitors block BMP9 signaling. So far, no selective ALK1 kinase inhibitors have been developed.

Table 1.

Effects of ALK1-targeting agents in pre-clinical studies.

Table 2.

Anti-ALK1 clinical trials in cancer (Clinicaltrials.gov)


TGF-β
Endoglin
TGF-β
BMP9
Endoglin
TGF-β
BMP9
TβRII
ALK5
TβRII
ALK5
ALK1
ALK1
BMPRII/ACTRII
Smad2/3
Smad2/3
P
Smad1/5/8
Smad1/5/8
P
Smad1/5/8
Smad4
Smad2/3
P
P
PAI-1
(CAGA12-luc)
Smad4
Smad1/5/8
P
P
Id1, Id3
(BRE-luc)


A
BMP9
PF-03446962
ALK1
ALK2
BMPRII/
ACTRII
B
Dalantercept
BMP9
ALK1
ALK2
BMPRII/
ACTRII
BMPRII/
ACTRII
C
BMP9
BMP9
ALK1
ALK2
BMPRII/
ACTRII
LDN-193189
K02288
LDN-193189
K02288

Table 1. Effects of ALK1-targeting agents in pre-clinical studies.

	Dalantercept	Ref	PF-3446962	Ref
Company	Acceleron pharma		Pfizer	
Description	ALK1-Fc fusion protein	*42*	anti-ALK1 antibody	*38*
Mode of action	binds to BMP9 and BMP10 (ligand trap)	*42*	binds to ALK1 extracellular domain (inhibits binding of all ligands that activate ALK1)	*38*
In vitro effects	blocks BMP9- and BMP10-induced Smad1 phosphorylation and downstream signaling	*42*	inhibits BMP9-induced Smad phosphorylation and BMP responsive element (BRE)-luciferase transcriptional reporter activity	*38*
	blocked human umbilical vein ECs (HUVEC) cord formation and endothelial sprouting and inhibited FGF-induced neovascularization and VEGF-induced vessel formation in a chick chorioallantoic membrane (CAM) assay	*42*	prevented binding of BMP9 to ECs, and blocked serum-induced Smad1 phosphorylation, migration and tube formation in HUVECs, as well as endothelial sprouting	*38, 40*
In vivo effects *Monotherapy*	MCF7 mammary adenocarcinoma orthotopic model: reduced tumor burden	*42*	MDA-MB-231 human breast cancer xenografts: reduction of tumor growth, inhibition of microvascular density and lymphatic vessel density	*40*
	RIP1-TAg2 pancreatic neuroendocrine model: reduction in vessel area (no increased hypoxia), increased pericyte coverage, impaired tumor growth and decreased number of hepatic micrometastases (long term treatment)	*24*		
	MMTV-PyMT and EO771 breast cancer models: reduced vessel area and delayed the growth and of primary mammary carcinomas and decreased the metastatic colonization of the lung	*44*	Chimera tumor model: reduction of human microvessel density	*40*
	Melanoma, head and neck and breast cancer models: no effect on primary tumor growth, reduction of vascular density and increased pericyte coverage	*45*		
In vivo effects *Combined therapy*	with the VEGFR2 neutralizing-antibody (DC101): not additional therapeutic benefit in MMTV-PyMT breast cancer model	*44*	with aVEGF RTK inhibitor (PF-00337210): enhanced tumor growth inhibition in M24met/R xenograft tumors, associated with enhanced reduction of $CD31^+$ vessels in the tumors, diminished $CD31^+$/$desmin^+$ costaining and increased sprouting	*40*
	with chemotherapy (Docetaxel): improved control of tumor growth, increased reduction of tumor vascularity of primary mammary carcinomas and decreased the metastatic colonization of the lung	*44*		
	with chemotherapy (Doxorubicin or Cisplatin): increased cytotoxic effect and impaired tumor growth in melanoma, head and neck and breast cancer models	*45*	with Bevacizumab: significant tumor growth inhibition compared to treatments alone, but no further reduction of human microvascular density (huMVD) in the chimera tumor model	*40*

Table 2. Anti-ALK1 clinical trials in cancer (Clinicaltrials.gov)

Drug	Clinical trial #	Phase	Condition	Start date	End date (or last updated)	Status	Ref
PF03446962	**NCT00557856**	1	Advanced Solid Tumors	November 2007	September 2015	Completed	36
PF03446962	**NCT01337050**	1	Neoplasms	April 2011	October 2015	Completed	
PF03446962	**NCT01486368**	2	Malignant Pleural Mesothelioma	December 2011	February 2015	Completed	
PF03446962	**NCT01911273**	2	Carcinoma, Hepatocellular	July 2013	October 2015	Terminated	
PF03446962	**NCT01620970**	2	Transitional Cell Carcinoma of Bladder	June 2012	June 2012	Unknown	
PF-03446962 and Regorafenib	**NCT02116894**	1b	Colorectal Cancer	April 2014	February 2016	Active, not recruiting	J Clin Oncol 34, 2016 (suppl; abstr e15013)
Dalantercept	**NCT00996957**	1	Advanced Solid Tumors Multiple Myeloma	October 2009	March 2013	Completed	37
Dalantercept	**NCT01458392**	2	Squamous cell carcinoma of the head and neck	October 2011	September 2015	Completed	
Dalantercept	**NCT01642082**	2	Endometrial Adenocarcinoma Endometrial Adenosquamous Carcinoma Endometrial Clear Cell Adenocarcinoma Endometrial Serous Adenocarcinoma Recurrent Uterine Corpus Carcinoma	July 2012	January 2016	Active, not recruiting	
Dalantercept	**NCT01720173**	2	Recurrent Fallopian Tube Carcinoma Recurrent Ovarian Carcinoma Recurrent Primary Peritoneal Carcinoma	October 2012	December 2014	Active, not recruiting	49 J Clin Oncol 34, 2016 (suppl; abstr e17050)
Dalantercept and axitinib	**NCT01727336**	2	Advanced Renal Cell Carcinoma	November 2012	September 2015	Active, recruiting	
Dalantercept plus sorafenib	**NCT02024087**	1b	Advanced Adult Hepatocellular Carcinoma	December 2013	May 2016	Active, not recruiting	

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Clinicaltrials.gov