

**TGF- $\beta$  & BMP receptors endoglin and ALK1: overview of their function and status as potential anti-angiogenic targets. [150 char max; currently 115]**

Running title: endoglin & ALK1 signalling and disease [40 char max; currently 39]

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**Abstract** [maximum of 200 words; currently 180]

The formation of new blood vessels from existing vasculature, angiogenesis, is facilitated through a host of different signalling processes. Members of the transforming growth factor-beta (TGF- $\beta$ ) superfamily, TGF- $\beta$ 1, TGF- $\beta$ 3, and BMP9, are key propagators of both inhibition and initiation of angiogenesis. Hereditary haemorrhagic telangiectasia (HHT), characterised by arteriovenous malformations and capillary bed defects, is caused by germline mutations in the *ENG* and *ACVRL1/ALK1* genes respectively. Clinical symptoms include epistaxis and gastrointestinal bleeds. The membranous receptors endoglin and ALK1 activate proliferation and migration of endothelial cells during the angiogenic process via downstream intracellular SMAD signalling pathway. Endothelial cell senescence or activation is dependent on the type of cytokine, ligand concentration, cell-cell interaction and a multitude of other signalling molecules. Endoglin and ALK1 receptor levels in tumour vasculature correlate inversely with prognosis in humans, whereas in mice, endoglin deficiency decelerates tumour progression. Therefore, endoglin and ALK1 have been identified as potential therapeutic targets for antibody treatment in various cancers. Early phase clinical trials in humans are currently underway to evaluate the efficacy and safety of biological therapy targeting endoglin/ALK1 mediated cells signalling.

**Keywords:** TGF- $\beta$ , BMP9, endoglin, ALK1, angiogenesis, vasculature, cancer

### List of abbreviations

<i>ACVRL1 / ALK1</i>	ALK-1 gene
ALK	Activin-like kinase
BMP	Bone morphogenetic protein
dpc	Days post-coitum
<i>ENG</i>	Endoglin gene
HHT	Hereditary haemorrhagic telangiectasia
HUVEC	Human umbilical vascular endothelial cells
TGF- $\beta$	Transforming growth factor- $\beta$

## Introduction

Since mutations in the genes encoding its receptors were first identified in patients with hereditary haemorrhagic telangiectasia in the mid-1990s, knowledge of the biological function of signalling receptor ALK1 co-receptor endoglin has increased dramatically. Both were originally annotated as transforming growth factor-beta (TGF- $\beta$ ) receptors (Cheifetz 1992; ten Dijke 1994), but this classification has since been challenged through data published by various research groups (Bailly 2008). Elucidation of TGF- $\beta$  family signalling involving endoglin and ALK1, particularly the near exclusive presence of both proteins in activated vascular endothelium (Goumans 2008), has led to pharmaceutical companies targeting this pathway for therapeutic purposes in oncology (Cunha 2011; Fonsatti 2010). In this review we summarise the unravelling of ALK1 and endoglin mediated TGF- $\beta$  superfamily signalling, and give a concise overview of the implications of changes in endoglin and ALK1 levels in disease and efforts to target their common signalling pathways in biological cancer therapy.

## TGF-beta signalling in vascular development

The TGF- $\beta$  cytokine family consists of a variety of signalling proteins including TGF- $\beta$ 1, 2 and 3, bone morphogenetic proteins (BMPs), activins, glial cell line-derived neurotrophic factors (GDNFs) and growth differentiation factors (GDFs) (Massague 2000). TGF- $\beta$  and BMP receptors ALK1 and endoglin are essential for embryonic vascular development.

Homozygous deficient *endoglin* and *Alk1* mouse embryos do not develop past mid-gestation due to lack of vascular growth in the yolk sac. Furthermore, TGF- $\beta$  type II receptor and ALK5 deficiency also leads to death in utero (Oshima 1996; Li 1999; Arthur 2000; Urness 2000).

Endoglin is expressed in mice as early as 6.5 days post-coitum (dpc), prior to the first

endothelial cells presenting in the mouse embryo (Jonker 2002). In humans, endoglin was found to be expressed almost exclusively in endothelium with transient upregulation of expression during cardiac development (Qu 1998).

Mouse gene knock-out models have shown that TGF- $\beta$ 1 deficiency leads to embryonic death at mid-gestation due to deficiencies in yolk sac vascular development, a phenotype akin to that seen in the receptor knockout mice (Dickson 1995; Oshima 1996; Li 1999; Arthur 2000; Urness 2000). The most recently identified ligand, whose signal is propagated into endothelium by endoglin/ALK1, is BMP9 (David 2007; Scharpfenecker 2007). Knockout mice deficient for BMP9 are viable and fertile without major abnormalities detected (Ricard, 2012). Since BMP9 and BMP10 are both capable of binding endoglin/ALK1, BMP10 may substitute for BMP9 in development (David 2007; Ricard 2012). This is feasible since BMP10 expression is restricted to embryogenesis (Chen 2004).

### **Hereditary haemorrhagic telangiectasia**

Mutations in the genes coding for endoglin and ALK1 proteins respectively, were identified as the underlying cause for the autosomal dominant disease hereditary haemorrhagic telangiectasia (HHT) in the mid-1990s. Type HHT1 is annotated to patients in whom *ENG* mutations are present, whereas *ALK1* (or *ACVRL1*) mutations lead to HHT2 (McAllister 1994, Johnson 1996). In rare cases other genes are implicated; *SMAD4*, whose protein is involved in TGF- $\beta$  signalling by being the common mediator for two divergent signalling pathways, is mutated in a syndrome characterised by gastrointestinal polyps and HHT-like symptoms (Gallione 2004). A fourth gene, whose product is implicated in TGF-beta signalling involving endoglin and ALK1 receptors, BMP type II receptor (BMPRII), is mutated in patients with

pulmonary hypertension. This phenotype is also a rare phenotype seen in HHT families (Trembath 2001; Upton 2009).

HHT has an incidence of approximately 1 in 5-8,000 (Porteau 1992; Kjeldsen 1999); clinical symptoms of HHT mostly involve recurrent epistaxis and arteriovenous malformations (AVM) develop in a proportion of circa 10-20% of patients, depending on the type of AVM.

HHT is clinically diagnosed using the Curacao criteria (Shovlin 2000), where at least three separate criteria are met: I) spontaneous recurrent epistaxis, II) mucocutaneous telangiectasia at typically common sites including fingertips, lips, oral mucosa or tongue, III) visceral involvement, such as gastrointestinal, pulmonary, hepatic, cerebral or spinal AVM, IV) family history: a first-degree relative affected according to these criteria. Although the Curacao criteria stipulate a presence of three characteristics, often a family history and an AVM are sufficient for diagnosis of HHT due to the rare occurrence of AVMs in the general population (Govani 2009).

Disease severity differs per patient and also within families, but the overall pathogenic mechanism for this heterozygous disorder is haploinsufficiency (Abdalla 2006). Various different types of mutations have been identified in both *endoglin* and *ALK1*; particularly in the case of endoglin, frameshift mutations occur most frequently ([www.hhtmud.org](http://www.hhtmud.org)). Some genotype-phenotype variation exists, with pulmonary and cerebral AVMs diagnosed more commonly in HHT1 and hepatic AVMs more prevalent in HHT2 (Letteboer 2006). However, overall mortality rates do not appear to differ between different genotypes (Kjeldsen 2005).

The recurrent epistaxis and the internal haemorrhage loss caused by gastrointestinal (GI) AVMs contribute to the morbidity burden of HHT. Patients will often require nasal packing and laser therapy for lesions in either the nose or GI tract, in addition to blood transfusions. Conversely, Pulmonary AVMs (PAVMs) are usually asymptomatic until the patient

experiences a stroke or brain abscess. Screening of HHT patients for PAVMS is therefore indicated, predominantly by performing thoracic CT scans, with embolization conducted when larger lesions are detected. Similar screening to PAVMs is in place for cerebral AVMs, and Doppler ultrasound screening can be used for hepatic AVMs. The clinical aspects of HHT are summarised in more detail in reviews by Govani & Shovlin (2009) and McDonald and colleagues (2011).

### **Canonical TGF-beta cell signalling**

The critical role of TGF- $\beta$  and its signalling receptors in development and cellular responses was eluded to earlier in this review. TGF- $\beta$  is unique in its ability to both inhibit and induce cell proliferation during developmental processes and in tissue homeostasis (Roberts 1981; Roberts 1986). Although most articles use the phrase TGF- $\beta$ , with this is generally meant TGF- $\beta$ 1, as is the case in this review. Together with TGF- $\beta$ 3, it targets the vascular endothelium in normal physiological circumstances to a greater extent than TGF- $\beta$ 2 (Jennings 1998; Merwin 1991). This is illustrated by the high levels of TGF-beta3 found in umbilical cord tissue (ten Dijke 1988). TGF- $\beta$ 2 was first identified in glioblastoma cells and is expressed in neural cells during embryonic development (Flanders 1991). In contrast to TGF- $\beta$ 1 and TGF- $\beta$ 3, which are targeted by co-receptor endoglin, TGF- $\beta$ 2 is bound to the co-receptor betaglycan (Lopez-Casillas 1994). In this manner, both auxiliary receptors promote presentation of the different TGF- $\beta$  ligands to the signalling receptor TGF- $\beta$  receptor II (T $\beta$ RII).

Target genes are reached by TGF- $\beta$  through a signalling cascade involving TGF- $\beta$  receptors and intracellular transcription factors called SMADs (Heldin 1997; Massague 2005). In cells where endoglin and ALK1 are not present, TGF- $\beta$  is bound by a heterotetrameric pair

consisting of a type I and type II TGF- $\beta$  receptor, ALK5 and T $\beta$ RII (REFERENCE). Post-translational modifications such as phosphorylation, ubiquitylation and sumoylation can influence the affinity for TGF- $\beta$  and the kinase activity of the receptor complex, which activates the downstream SMAD proteins (Heldin 1997). TGF- $\beta$ , a dimer protein complex, is itself not readily active and requires activation from its latent form by other molecules such as retinoic acid and integrins (Annes 2003).

Suppression of cell proliferation is a core effect of TGF- $\beta$  in most cell types; epithelium being an example. Since expression of ALK1 and endoglin is restricted primarily to the endothelium, the tumour suppressor effect of TGF- $\beta$  is therefore achieved in most cells of a healthy adult person (Derynck 2001). The downstream activated SMADs can suppress expression of c-Myc and cyclin-dependent kinases (CDKs) and increase expression of CDK inhibitors p15<sup>INK4B</sup> and p27<sup>KIP1</sup> and achieve cell senescence in this manner (REFERENCE). In the case of T $\beta$ RII and ALK5 complex signalling, the common mediator SMAD4 (co-SMAD) will form a complex with a receptor-regulated SMAD (r-SMAD) that has been activated through phosphorylation by the receptor complex (Heldin 1997). In the case of ALK5, only the rSMADs SMAD2 and SMAD3 can be activated (REFERENCE). The complex, which can consist of three proteins such as SMAD4 and two of phosphorylated SMAD2 and/or SMAD3 in one complex, will then exert its transcriptional activity. Transcriptional activity will depend on the cell type and also on the presence of co-activators and co-repressors (see for example Massague 2005 for in-depth review). In contrast to r-SMADs, inhibitory SMADs (i-SMADs) will block the above process by binding to the TGF- $\beta$  type I receptor and thereby hindering r-SMAD from being activated (REFERENCE). SMADs have well conserved domains which explain how they can compete. Regulatory SMADs (SMAD1, SMAD2, SMAD3, SMAD5, SMAD8) and the co-SMAD have both a Mad-homology-1 (MH1) domain to bind DNA and a



MH2 domain to bind the TGF-beta receptors, whereas i-SMADS (SMAD6 and SMAD7) only contain the MH2 domain (REFERENCE). Apart from the canonical SMAD signalling pathway for TGF- $\beta$ , a host of other non-SMAD signalling pathways via TGF- $\beta$  receptors have been identified, such as mitogen-activated kinases (MAPK) pathways (Derynck 2003; Zhang 2008). Combinations of available receptors, a mixture of TGF- $\beta$  and other cytokines in various concentrations, and activation of 'core' SMAD and divergent MAPK pathways influence the way a cell will behave.

### **TGF-beta & BMP9 signalling via endoglin and ALK1**

The homodimeric endoglin protein has a very small intracellular domain lacking kinase activity, and therefore plays a modulating role, whereas ALK1 phosphorylates target r-SMADS (SMAD1, SMAD5 and SMAD8) in the same manner as ALK5 (REFERENCE). This in turn leads to transcription of different genes compared to those induced by the ALK5 targets, SMAD2 and SMAD3. In immortalised mouse endothelial cells, the presence of endoglin and ALK1 on the cell surface allows TGF-beta to activate SMAD1/5/8, which can also be activated by BMP cytokines (Suzuki 2010; David 2007; Scharpfenecker 2007). Both the ALK1 and ALK5 mediated signalling pathways can be activated within endothelium (reviewed by Goumans 2008). Although not a signalling receptor in its own right, endoglin is a key element for effective TGF- $\beta$  signalling via ALK1 (Lebrin 2004; Carvalho 2004). Not only does endoglin interact with ALK1 to promote downstream SMAD1/5/8 activation, it also actively disrupts ALK5 mediated signalling (Goumans 2003; Blanco 2005) .

The requirement of endoglin for endothelial cell proliferation and survival is illustrated by the fact that endothelial cells lines could not be derived from homozygous endoglin deficient mouse embryonic endothelial cells (Lebrin 2004). Conversely, overexpression of

both endoglin and ALK1 increases cell proliferation. Introduction of a kinase-deficient ALK1 protein also had a detrimental effect on endothelial cell proliferation (Lebrin 2004). There is conflicting published data available concerning the exact downstream r-SMAD targets for endoglin/ALK1, but evidence outlined below supports a model where it is mainly BMP9 signal that is transduced by endoglin/ALK1 in vivo, rather than TGF-beta. Nevertheless, it has been shown that TGF- $\beta$ 1 added to endoglin deficient mouse endothelial cells in vitro does not affect Smad2 phosphorylation but fails to induce Smad1/5/8 phosphorylation; presence of normal endoglin protein reverses this effect (Lebrin 2004).

Where, for TGF-beta, the net effect on endothelial cells can be partially explained in an experimental setting by the presence of either ALK5 or endoglin/ALK1, conflicting outcomes have been observed for BMP9 in vitro. In one experiment, human primary endothelial cells (HUVEC) were exposed to TGF-beta, and SMAD1/5/8 proteins were not activated. Unlike with TGF-beta, exposure of the same cells with BMP9 did lead to SMAD1/5/8 phosphorylation (Nolan-Stevaux 2012). Anti-ALK1 antibody interferes with both BMP9 and TGF-beta signalling in vitro in HUVECs, and reduction of endoglin and ALK1 mRNA affects expression levels of the BMP9 downstream target stromal derived factor 1 (SDF1), a protein induced in hypoxia and known to promote vascular remodelling (van Meeteren 2012; Young 2012). Akin to TGF-beta's pro-angiogenic effect when processed through endoglin/ALK1, BMP9 was shown to signal via endoglin/ALK1 and promote in vitro angiogenesis in the study by Nolan-Stevaux (2012). Proliferation of endothelial cells can also be induced by BMP9 (Suzuki 2010). Earlier, exposure of human microvascular endothelial cells in vitro with BMP9 and BMP10 was shown to lead to ALK1 and downstream SMAD1/5/8 activation, reversed by suppression of ALK1 expression and enhanced by overexpression of endoglin (Scharpfenecker 2007; David 2007). In contrast, Scharpfenecker (2007) and colleagues

observed an anti-angiogenic response when bovine aortic endothelial cells (BAECs) were exposed to BMP9, and similar data has been obtained in murine endothelial cells (Scharpfenecker 2007; David 2008).

The abovementioned studies have clearly demonstrated a vital function for BMP9 in angiogenesis as observed through in vitro and in vivo experiments. Furthermore, BMP9 is present and active in human serum (David 2008). Yet, the pro- and anti-angiogenic roles of BMP9 are still up for debate. The conflicting results may be borne from the different type II receptors that ALK1 may link up with to form a heterotetrameric complex; when processing BMP9 it can bind either activin receptor type II B (ActRIIB) or BMPRIIB (Townson 2012). Overall functional importance of endoglin/ALK1 signalling for BMP10 is less certain, since BMP10 expression is restricted to cardiac tissue during embryogenesis (David 2007). Figure 1 provides a simplified outline of the respective TGF- $\beta$  and BMP9 signalling pathways in endothelium.

### **Biological therapy to inhibit angiogenesis**

Endoglin is expressed in human endothelium of developing and mature vessels, as well as being expressed at high levels in endothelium undergoing angiogenesis in normal physiological processes, wound healing, and within tumours (Torsney ; Goumans 2008). In line with the data obtained in mouse tissue, increased endoglin levels are associated with higher levels of endothelial cell proliferation (Lebrin 2004). As a result of these characteristics, endoglin is used as a marker for tumour angiogenesis (Fonsatti 2000, Bredow 2000). With the rise of antibody (biologicals) therapy to target specific proteins in tumour tissue, and the presence of endoglin in the blood vessels of tumours, the potential

for endoglin to be targeted for anti-angiogenic therapy in cancer was already recognised in the late 1990s (Seon 1997, Takahashi 2001).

Endoglin expression not only correlates with levels of angiogenesis in tumours; protein levels also negatively correlate with overall survival, disease-free survival and presence of metastases in different types of cancers (Kumar 1999; Brewer 2000). Furthermore, oncological staging of different types of cancer also correlate with endoglin levels, like in the case of the Gleason score for prostate carcinoma (Wikstrom 2000). In vivo experiments in mice, involving inoculation of Lewis lung carcinoma cells into wild-type and heterozygous mutant endoglin mice supported not only the critical role of endoglin in initiating angiogenesis but also the need for endoglin to allow tumour vascularisation and growth (Duwel 2007). **ALK1 deficiency, when targeted through genetic or pharmacological methodology, (Cunha 2010).** Other reviews have earlier covered the topic of anti-angiogenesis for endoglin (Fonsatti 2010) and ALK1 (Cunha 2011; Vecchia 2013) respectively. Below the three investigational medicinal products to target endoglin and ALK1 function are presented. Current anti-angiogenic treatment for cancer patients, like bevacizumab, sorafenib, sunitib, and pazopanib, target VEGF signalling and patient survival has on average only improved moderately with resistance to therapy an issue for various cancers (Ellis & Hicklin, 2008; Rini & Atkins 2009; Bergers&Hanahan 2008). The potential therapeutic agents below may therefore in the future be trialled as a sole treatment or as an adjunct to chemotherapy and/or anti-VEGF therapy.

## **TCR105**

The chimeric immunoglobulin G1 (IgG1) monoclonal antibody TRC105, developed by TRACON Pharmaceuticals, binds endoglin (Rosen 2012). Its murine originator, SN6j, and

TRC105 itself inhibit tumor progression and angiogenesis in mice, and can induce apoptosis in endothelial cells in vitro (Uneda 2009; Seon 2011). A first-in-human phase I trial in the United States involving 50 patients with non-curable cancer, were given 10 mg/kg/week or 15 mg/kg/ 2 weeks (Rosen 2012). At time of reporting, two patients remained on treatment for at least 48 and 18 months, respectively, with a fraction of patients being progression-free at four months (6 out of 44 patients). Thirty-three patients stopped treatment due to disease progression. Eight patients discontinued due to adverse events, with 15 mg/kg/2 weeks causing grade 3 or 4 hypoproliferative anemia; grade 3 also developed in one patient on the 10 mg/kg/week dosage schedule. Infusion reactions did occur with TRC105 treatment. However, this is a common adverse event for treatment with biologicals (Campi 2007). Other adverse events of note were the development of telangiectasia on the trunk of 3 patients, and occurrence of grade 4 GI haemorrhage from a gastric ulcer. This prompted the addition of peptic ulcer disease to the exclusion criteria of the trial protocol, in addition to exclusion of those patients with a marked cardiovascular disease, history of haemorrhage, unhealed surgical wounds, and pregnancy from the onset of the study (Rosen 2012).

### **PF-3446962**

The completely human IgG2 monoclonal antibody PF-3446962 is being developed by Pfizer and targets ALK1 (Goff 2010). In mouse and human models of angiogenesis and tumorigenesis, PF-03446962 suppresses both processes (Hu-Lowe 2011). A phase I study involving patients with solid tumours is underway to find an optimal dose for follow-up phase II trials (Goff 2010). Results disclosed to date include a maximum dose applied of up to 6.75 mg/kg, with data available up to a dosage of 4.5 mg/kg, and no grade 3 or 4 adverse

events observed. In parallel to using PF-03446962 as a therapeutic agent, Pfizer is also evaluating the use of this antibody as a biomarker for detection of ALK1-positive circulating endothelial cells (CEC). Early work has identified higher levels of ALK1 expressed on CECs of cancer patients, and a higher incidence of CECs in blood of cancer patients compared to healthy subjects (Mancuso 2001, Mancuso 2009), This may allow development of an assay to identify patients suitable for anti-ALK1 therapy and/or therapy response (Mancuso 2009).

### **Dalantercept (ACE-041)**

Fusion protein ACE-041 contains of the extracellular domain of human ALK1 linked to the Fc portion of human IgG1 and is being developed by Acceleron Pharma under the non-proprietary name dalantercept (Borgstein 2010). ACE-041 functions as a soluble trap for BMP9 and BMP10, thereby blocking downstream signalling via ALK1; it does not block TGF-beta (Cunha 2010; Mitchell 2010)

One phase I trial update has been published, which outlines the patient group and tumour types recruited – solid tumours and refractory multiple myeloma. Outcome measures include safety and tolerability and changes in tumour metabolism (Borgstein, 2010). Signs of toxicity were observed, such as edema and fluid overload.

### **Safety of anti-endoglin & anti-ALK1 therapy**

Endoglin and ALK1 not only play a role in angiogenesis in tumours, but also in vascular remodelling and inflammatory processes in adult mammals and therefore caution is warranted in relation to trials of biologicals that systemically inhibit endoglin, ALK1 or BMP9. Endoglin expression is up-regulated during wound healing, and also plays a role in the menstrual cycle in females (Kim 2001; Torsney 2002). Likewise, ALK1 has been shown to be induced during wound healing (Seki 2003). Cutaneous wounds inflicted on both heterozygous *endoglin* mutant and wild-type mice heal slower in the former, which was at

least partly caused by less nitric oxide being available (Perez-Gomez 2013). Changes in TGF- $\beta$  signalling via endoglin/ALK1 at the site of the wound are not short-term; in humans higher levels of endoglin persist for up to a month (Torsney 2002). In females, endoglin is expressed in the arterial vessels of the endometrium, particularly during the late secretory phase and therefore may contribute to maintenance of vessel integrity function (Kim 2001; Zhang 2002). Vascularisation of the placenta, in mice at least and therefore conceivably in humans too, is also regulated via ALK1 (Hong 2007).

Although beyond the scope of this review, in disease processes other than neoangiogenesis in cancer, such as pre-eclampsia and hepatic fibrosis, endoglin and ALK1 play functional roles (Levine 2006; Wiercinska 2006). This illustrates that, in a cancer patient, endoglin/ALK1 signalling of TGF- $\beta$  and BMP9 is not necessarily confined to the tumour environment. Theoretically, persistent systemic inhibition of endoglin or ALK1 may impact on wound healing, and potentially cause intermenstrual bleeding. Since haploinsufficiency is the pathogenic mechanism for HHT, clinical researchers involved in trials concerned with inhibition of endoglin or ALK1 signalling are bound to be vigilant for adverse events related to phenotypes seen in HHT patients, including recurrent epistaxis, arteriovenous shunts and pulmonary (arterial) hypertension.

## **Conclusion**

Whilst numerous phase I and II trials are underway, further work is warranted to dissect signalling via endoglin and ALK1 at the molecular level. It remains a challenge to determine the exact extent to which TGF- $\beta$  and BMP9 signal are propagated in vivo by endoglin/ALK1. Progress is complicated by the multifaceted nature of TGF- $\beta$  superfamily signalling and the

added complexity of other cytokine families that can have an impact on endothelial cell behaviour, such as VEGF. Interaction between endothelium and components in both blood and underlying tissue such as stroma, smooth muscle cells and tumour cells can also influence physiological outcomes. The exact role of BMP9 in mammal adulthood is not yet fully understood and warrants investigation in light of steps taken to systemically inhibit it with Dalantercept. The potential of anti-endoglin (with TCR105) and anti-ALK1 (using PF-3446962) therapy to target neovascularisation in cancer patients is clear since endoglin and ALK1 are expressed primarily in active vascular endothelium. It will be intriguing to see whether the effectiveness of targeting this pathway outweighs any potential side effects. Some hallmark symptoms of HHT, like telangiectasia, have been detected in patients undergoing the first trials with these novel anti-angiogenic biologicals (Rosen 2012; Bendell 2011; Goff 2011).

## **Perspectives**

The TGF- $\beta$  receptors endoglin and ALK1 transduce TGF- $\beta$  and BMP9 cytokine signalling to promote activation and proliferation of endothelial cells in processes including angiogenesis. Due to their expression profiles, both endoglin and ALK1 antibody therapy are being trialled for treatment of various types of cancer. However, the phenotype of the genetic disorder hereditary haemorrhagic telangiectasia, with symptoms such as recurrent epistaxis and arteriovenous malformations due to haploinsufficiency, and the roles of endoglin and ALK1 in wound healing mean there is a potential for adverse effects.



## References

Heldin, C. H., Miyazono, K., & Ten Dijke, P. (1997). TGF- $\beta$  signalling from cell membrane to nucleus through SMAD proteins. *Nature*, 390(6659), 465-471.

Derynck, R., Akhurst, R. J., & Balmain, A. (2001). TGF- $\beta$  signaling in tumor suppression and cancer progression. *Nature genetics*, 29(2), 117-129.

Goumans, Marie-José, Gudrun Valdimarsdottir, Susumu Itoh, Franck Lebrin, Jonas Larsson, Christine Mummery, Stefan Karlsson, and Peter ten Dijke. "Activin receptor-like kinase (ALK) 1 is an antagonistic mediator of lateral TGF $\beta$ /ALK5 signaling." *Molecular cell* 12, no. 4 (2003): 817-828.

Cheifetz, S., Bellon, T., Cales, C., Vera, S., Bernabeu, C., Massague, J., & Letarte, M. (1992). Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *Journal of Biological Chemistry*, 267(27), 19027-19030.

Massagué, J., Blain, S. W., & Lo, R. S. (2000). TGF $\beta$  signaling in growth control, cancer, and heritable disorders. *Cell*, 103(2), 295-309.

Goumans, M. J., Liu, Z., & ten Dijke, P. (2008). TGF- $\beta$  signaling in vascular biology and dysfunction. *Cell research*, 19(1), 116-127.

Ten Dijke, P., Yamashita, H., Ichijo, H., Franzen, P., Laiho, M., Miyazono, K., & Heldin, C. H. (1994). Characterization of type I receptors for transforming growth factor-beta and activin. *Science*, 264(5155), 101-104.

Carvalho, Rita LC, Leon Jonker, Marie-José Goumans, Jonas Larsson, Peter Bouwman, Stefan Karlsson, Peter ten Dijke, Helen M. Arthur, and Christine L. Mummery. "Defective paracrine signalling

by TGF $\beta$  in yolk sac vasculature of endoglin mutant mice: a paradigm for hereditary haemorrhagic telangiectasia." *Development* 131, no. 24 (2004): 6237-6247.

Jennings, J. C., Mohan, S., Linkhart, T. A., Widstrom, R., & Baylink, D. J. (1988). Comparison of the biological actions of TGF beta-1 and TGF beta-2: Differential activity in endothelial cells. *Journal of cellular physiology*, 137(1), 167-172.

López-Casillas, F., Payne, H. M., Andres, J. L., & Massagué, J. (1994). Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. *The Journal of cell biology*, 124(4), 557-568.

Merwin, J. R., Roberts, A., Kondaiah, P., Tucker, A., & Madri, J. (1991). Vascular cell responses to TGF- $\beta$ 3 mimic those of TGF- $\beta$ 1 in vitro. *Growth Factors*, 5(2), 149-158.

Campi, P., Benucci, M., Manfredi, M., & Demoly, P. (2007). Hypersensitivity reactions to biological agents with special emphasis on tumor necrosis factor-[alpha] antagonists. *Current opinion in allergy and clinical immunology*, 7(5), 393-403.

1. Annes, Justin P., John S. Munger, and Daniel B. Rifkin. "Making sense of latent TGF $\beta$  activation." *Journal of cell science* 116, no. 2 (2003): 217-224.
2. Arthur, H. M., Ure, J., Smith, A. J., Renforth, G., Wilson, D. I., Torsney, E., ... & Diamond, A. G. (2000). Endoglin, an ancillary TGF $\beta$  receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Developmental biology*, 217(1), 42-53.
3. Bailly, S. (2008). HHT is not a TGF $\beta$  disease. *Blood*, 111(2), 478-478.
4. Bendell, J.C. Gordon M.S., Hurwitz H., Condon C.H., Wilson D., Yang Y., et al. A Phase I dose escalation study of ACE-041, a novel inhibitor of ALK1-mediated angiogenesis,

in patients with advanced solid tumors, in American Society of Clinical Oncology  
2011: Chicago, IL.

5. Bergers, G. & Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat rev Cancer* 8:592-603, 2008.
6. Borgstein NG, Sharma S, Bendell J.C., et al. Phase I study of ace-041, a novel inhibitor of vascular maturation in patients with advanced solid tumours or relapsed/refractory multiple myeloma. *J Clin Oncol (Meeting Abstracts)*. 28(15 suppl): TPS179, 2010.
7. Brewer, C. A., Setterdahl, J. J., Li, M. J., Johnston, J. M., Mann, J. L., & McAsey, M. E. (2000). Endoglin expression as a measure of microvessel density in cervical cancer. *Obstetrics & Gynecology*, 96(2), 224-228.
8. Chen, Hanying, Shu Shi, Lourdes Acosta, Weiming Li, Jonathan Lu, Shideng Bao, Zhuang Chen et al. "BMP10 is essential for maintaining cardiac growth during murine cardiogenesis." *Development* 131, no. 9 (2004): 2219-2231.
9. Cunha S.I and Pietras K. ALK1 as an emerging target for antiangiogenic therapy of cancer. *Blood* 117: 6999-7006, 2011.
10. Cunha S.I., Pardali E., Thorikay M., et al. Genetic and pharmacological targeting of activin receptor-like kinase 1 impairs tumor growth and angiogenesis. *J Exp Med*. 207:85-100, 2010
11. Cunha, Sara I., Evangelia Pardali, Midory Thorikay, Charlotte Anderberg, Lukas Hawinkels, Marie-José Goumans, Jasbir Sehra, Carl-Henrik Heldin, Peter ten Dijke, and Kristian Pietras. "Genetic and pharmacological targeting of activin receptor-like kinase 1 impairs tumor growth and angiogenesis." *The Journal of experimental medicine* 207, no. 1 (2010): 85-100.

12. David, L., Mallet, C., Mazerbourg, S., Feige, J. J., & Bailly, S. (2007). Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood*, 109(5), 1953-1961.
13. David, Laurent, Christine Mallet, Michelle Keramidas, Noël Lamandé, Jean-Marie Gasc, Sophie Dupuis-Girod, Henri Plauchu, Jean-Jacques Feige, and Sabine Bailly. "Bone morphogenetic protein-9 is a circulating vascular quiescence factor." *Circulation research* 102, no. 8 (2008): 914-922.
14. Derynck, R., & Zhang, Y. E. Smad-dependent and Smad-independent pathways in TGF- $\beta$  family signalling. *Nature*, 425: 577-584, 2003.
15. Dickson, M. C., Martin, J. S., Cousins, F. M., Kulkarni, A. B., Karlsson, S., & Akhurst, R. J. (1995). Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development*, 121(6), 1845-1854.
16. Düwel, A., Eleno, N., Jerkic, M., Arevalo, M., Bolaños, J. P., Bernabeu, C., & Lopez-Novoa, J. M. (2006). Reduced tumor growth and angiogenesis in endoglin-haploinsufficient mice. *Tumor Biology*, 28(1), 1-8.
17. Ellis L.M & Hicklin D.J. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat rev Cancer*. 8:579-591, 2008.
18. Flanders, KATHLEEN C., G. E. R. S. O. N. Ludecke, S. I. B. E. L. L. E. Engels, DAVID S. Cissel, ANITA B. Roberts, P. A. T. U. R. U. Kondaiah, R. O. B. E. R. T. Lafyatis, MICHAEL B. Sporn, and K. L. A. U. S. Unsicker. "Localization and actions of transforming growth factor-beta s in the embryonic nervous system." *Development* 113, no. 1 (1991): 183-191.

19. Fonsatti E, Nicolay H.J.M. altomonte M., Covre A., Maio M. Targeting cancer vasculature via endoglin/CD105: a novel antibody-based diagnostic and therapeutic strategy in solid tumours. *Cardiovasc. Res.* 86: 12-19, 2010.
20. Fonsatti, Ester, Antti P. Jekunen, Kalevi JA Kairemo, Sandra Coral, Marjatta Snellman, Maria Rita Nicotra, Pier Giorgio Natali, Maresa Altomonte, and Michele Maio.  
"Endoglin is a suitable target for efficient imaging of solid tumors: in vivo evidence in a canine mammary carcinoma model." *Clinical Cancer Research* 6, no. 5 (2000): 2037-2043.
21. Goff L.W., Cohen R.B, Berlin J., Noberasco C., Borghaei H., Gallo-Stampino C. et al.  
Phase I study of PF-03446962, a fully human mAb against ALK1, a TGF-beta receptor involved in tumor angiogenesis, in American Society of Clinical Oncology 2011: Chicago, IL.
22. Goff, L. W., F. G. De Braud, R. B. Cohen, J. Berlin, C. Noberasco, H. Borghaei, E. Wang, D. Hu Lowe, W. J. Levin, and C. Gallo-Stampino. "Phase I study of PF-03446962, a fully human mab against ALK 1, a TGF {beta} receptor involved in tumor angiogenesis." In *J Clin Oncol (Meeting Abstracts)*, vol. 28, no. 15 suppl, p. 3034. 2010.
23. Govani, F. S., & Shovlin, C. L. Hereditary haemorrhagic telangiectasia: a clinical and scientific review. *European Journal of Human Genetics*, 17: 860-871, 2009.
24. Hong, K. H., Seki, T., & Oh, S. P. (2007). Activin receptor-like kinase 1 is essential for placental vascular development in mice. *Laboratory investigation*, 87(7), 670-679.
25. Hu-Lowe, Dana D., Enhong Chen, Lianglin Zhang, Katherine D. Watson, Patrizia Mancuso, Patrick Lappin, Grant Wickman et al. "Targeting activin receptor-like kinase 1 inhibits angiogenesis and tumorigenesis through a mechanism of action

complementary to anti-VEGF therapies." *Cancer research* 71, no. 4 (2011): 1362-1373.

26. Johnson, D. W., J. N. Berg, M. A. Baldwin, C. J. Gallione, I. Marondel, S-J. Yoon, T. T. Stenzel et al. "Mutations in the activin receptor–like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2." *Nature genetics* 13, no. 2 (1996): 189-195.
27. Jonker, L., & Arthur, H. M. (2002). Endoglin expression in early development is associated with vasculogenesis and angiogenesis. *Mechanisms of development*, 110(1), 193-196.
28. Kim, S. H., Choi, Y. M., Chae, H. D., Kim, K. R., Kim, C. H., & Kang, B. M. (2001). Increased expression of endoglin in the eutopic endometrium of women with endometriosis. *Fertility and sterility*, 76(5), 918-922.
29. Kjeldsen, A. D., Møller, T. R., Brusgaard, K., Vase, P., & Andersen, P. E. (2005). Clinical symptoms according to genotype amongst patients with hereditary haemorrhagic telangiectasia. *Journal of internal medicine*, 258(4), 349-355.
30. Kumar, S., Ghellal, A., Li, C., Byrne, G., Haboubi, N., Wang, J. M., & Bundred, N. (1999). Breast carcinoma vascular density determined using CD105 antibody correlates with tumor prognosis. *Cancer Research*, 59(4), 856-861.
31. Lebrin, Franck, Marie-Jose Goumans, Leon Jonker, Rita LC Carvalho, Gudrun Valdimarsdottir, Midory Thorikay, Christine Mummery, Helen M. Arthur, and Peter ten Dijke. "Endoglin promotes endothelial cell proliferation and TGF- $\beta$ /ALK1 signal transduction." *The EMBO journal* 23, no. 20 (2004): 4018-4028.
32. Letteboer, T. G., Mager, J. J., Snijder, R. J., Koeleman, B. P., Lindhout, D., van Amstel, J. P., & Westermann, C. J. J. (2006). Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. *Journal of medical genetics*, 43(4), 371-377.

33. Levine, Richard J., Chun Lam, Cong Qian, Kai F. Yu, Sharon E. Maynard, Benjamin P. Sachs, Baha M. Sibai et al. "Soluble endoglin and other circulating antiangiogenic factors in preeclampsia." *New England Journal of Medicine* 355, no. 10 (2006): 992-1005.
34. Mancuso, P., Burlini, A., Pruneri, G., Goldhirsch, A., Martinelli, G., & Bertolini, F. (2001). Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood*, 97(11), 3658-3661.
35. Mancuso, P., D. R. Shalinsky, A. Calleri, J. Quarna, P. Antoniotti, I. Jilani, D. Hu-Lowe, X. Jiang, C. Gallo-Stampino, and F. Bertolini. "Evaluation of ALK-1 expression in circulating endothelial cells (CECs) as an exploratory biomarker for PF-03446962 undergoing phase I trial in cancer patients." In *J Clin Oncol (Meeting Abstracts)*, vol. 27, no. 15S, p. 3573. 2009.
36. Massagué, J., Seoane, J., & Wotton, D. (2005). Smad transcription factors. *Genes & development*, 19(23), 2783-2810.
37. McAllister, K. A., K. M. Grogg, D. Wetall Johnson, C. J. Gallione, M. A. Baldwin, C. E. Jackson, E. A. Helmbold et al. "Endoglin, a TGF- $\beta$  binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1." *Nature genetics* 8, no. 4 (1994): 345-351.
38. Mitchell D., Pobre E.g., MulivorA.W., et al. Alk1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. *Mol Cancer ther* 9:379-388, 2010.
39. Nolan-Stevaux, O., Zhong, W., Culp, S., Shaffer, K., Hoover, J., Wickramasinghe, D., & Ruefli-Brasse, A. (2012). Endoglin Requirement for BMP9 Signaling in Endothelial Cells Reveals New Mechanism of Action for Selective Anti-Endoglin Antibodies. *PloS one*, 7(12), e50920.

40. Oshima, M., Oshima, H., & Taketo, M. M. (1996). TGF- $\beta$  receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Developmental biology*, 179(1), 297-302.
41. Pérez-Gómez E, Jerkic M, Prieto M, Del Castillo G, Martín-Villar E, Letarte M, Bernabeu C, Pérez-Barriocanal F, Quintanilla M, López-Novoa JM Impaired Wound Repair in Adult Endoglin Heterozygous Mice Associated with Lower NO Bioavailability. *J Invest Dermatol*. 2013 Jun 13. doi: 10.1038/jid.2013.263. [Epub ahead of print]
42. Porteous, M. E., Burn, J., & Proctor, S. J. (1992). Hereditary haemorrhagic telangiectasia: a clinical analysis. *Journal of medical genetics*, 29(8), 527-530.
43. Qu, R., Silver, M. M., & Letarte, M. (1998). Distribution of endoglin in early human development reveals high levels on endocardial cushion tissue mesenchyme during valve formation. *Cell and tissue research*, 292(2), 333-343.
44. Ricard, Nicolas, Delphine Ciais, Sandrine Levet, Mariela Subileau, Christine Mallet, Teresa A. Zimmers, Se-Jin Lee, Marie Bidart, Jean-Jacques Feige, and Sabine Bailly. "BMP9 and BMP10 are critical for postnatal retinal vascular remodeling." *Blood* 119, no. 25 (2012): 6162-6171.
45. Rini B.I. & Atkins M.B. Resistance to targeted therapy in renal-cell carcinoma. *Lancet Oncology* 10: 992-1000, 2009.
46. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB (1981) New class of transforming growth factors potentiated by epidermal growth factor. *Proc Natl Acad Sci USA* 78: 5339–5343
47. Roberts, Anita B., Michael B. Sporn, Richard K. Assoian, Joseph M. Smith, Nanette S. Roche, Lalage M. Wakefield, Ursula I. Heine, Lance A. Liotta, Vincent Falanga, and



John H. Kehrl. "Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro." *Proceedings of the National Academy of Sciences* 83, no. 12 (1986): 4167-4171.

48. Rosen L.S., Hurwitz H. I., Wong, M.K.K. A phase 1 first-in-human study of TRC105 (anti-endoglin antibody) in patients with advanced cancer. Clin Cancer Res. Do:10.1158/1078-0432.CCR-12-0098
49. Scharpfenecker M, van Dinther M, Liu Z, van Bezooijen R.L., Qinghai Zhao, Laurie Pukac, Clemens WGM Löwik, ten Dijke P. "BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis." *Journal of cell science* 120, no. 6 (2007): 964-972.
50. Seki, T., Yun, J., & Oh, S. P. (2003). Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterialization and vascular remodeling. *Circulation research*, 93(7), 682-689.
51. Seon B.K., Haba A., Matsuno F., Takahashi N., Tsujie M., She X., et al. Endoglin-targeted cancer therapy. *Curr Drug Deliv* 8: 135-143, 2011.
52. Seon, B. K., Matsuno, F., Haruta, Y., Kondo, M., & Barcos, M. (1997). Long-lasting complete inhibition of human solid tumors in SCID mice by targeting endothelial cells of tumor vasculature with antihuman endoglin immunotoxin. *Clinical cancer research*, 3(7), 1031-1044.
53. Shovlin, Claire L., Guttmacher A.E., Buscarini E., Faughnan M.E., Hyland R.H., Westermann C.J.J., Kjeldsen A.D., Plauchu H.. "Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome)." *American journal of medical genetics* 91: 66-67, 2000.

54. Suzuki, Y., Ohga, N., Morishita, Y., Hida, K., Miyazono, K., & Watabe, T. (2010). BMP-9 induces proliferation of multiple types of endothelial cells in vitro and in vivo. *Journal of cell science*, 123(10), 1684-1692.
55. ten Dijke P, Hansen P, Iwata KK, Pieler C, Foulkes JG. Identification of another member of the transforming growth factor type beta gene family. *Proc Natl Acad Sci U S A*. 1988 Jul;85(13):4715-9.
56. Torsney E., Charlton R., Parums D., Collis M., Arthur H.M.. "Inducible expression of human endoglin during inflammation and wound healing in vivo." *Inflammation Research* 51, no. 9 (2002): 464-470.
57. Townson, S. A., Martinez-Hackert, E., Greppi, C., Lowden, P., Sako, D., Liu, J., ... & Grinberg, A. V. (2012). Specificity and structure of a high affinity activin receptor-like kinase 1 (ALK1) signaling complex. *Journal of Biological Chemistry*, 287(33), 27313-27325.
58. Trembath, R.C., Thomson J.R., Rajiv D. Machado, Neil V. Morgan, Carl Atkinson, Ingrid Winship, Gerald Simonneau et al. "Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia." *New England Journal of Medicine* 345, no. 5 (2001): 325-334.
59. Uneda S, Toi H., Seon B.K. Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature. *Int J Cancer* 125: 1446-1453, 2009.
60. Upton, P. D., Davies, R. J., Trembath, R. C., & Morrell, N. W. (2009). Bone morphogenetic protein (BMP) and activin type II receptors balance BMP9 signals mediated by activin receptor-like kinase-1 in human pulmonary artery endothelial cells. *Journal of Biological Chemistry*, 284(23), 15794-15804.

61. Urness, L. D., Sorensen, L. K., & Li, D. Y. (2000). Arteriovenous malformations in mice lacking activin receptor-like kinase-1. *Nature genetics*, 26(3), 328-331.
62. van Meeteren, Laurens A., Midory Thorikay, Simon Bergqvist, Evangelia Pardali, Corrado Gallo Stampino, Dana Hu-Lowe, Marie-José Goumans, and Peter ten Dijke. "Anti-human activin receptor-like kinase 1 (ALK1) antibody attenuates bone morphogenetic protein 9 (BMP9)-induced ALK1 signaling and interferes with endothelial cell sprouting." *Journal of Biological Chemistry* 287, no. 22 (2012): 18551-18561.
63. Vecchia L, Olivieri C, Scotti C. Activin receptor-like kinase 1: a novel anti-angiogenesis target from TGF-beta family. *Mini Rev Med Chem*. 2013 Jun 26 [epub ahead of print].
64. Wiercinska, Eliza, Lucia Wickert, Bernd Denecke, Harun M. Said, Jafar Hamzavi, A. M. Gressner, Midori Thorikay et al. "Id1 is a critical mediator in TGF- $\beta$ -induced transdifferentiation of rat hepatic stellate cells." *Hepatology* 43, no. 5 (2006): 1032-1041.
65. Wikström, P., Lissbrant, I. F., Stattin, P., Egevad, L., & Bergh, A. (2002). Endoglin (CD105) is expressed on immature blood vessels and is a marker for survival in prostate cancer. *The Prostate*, 51(4), 268-275.
66. Zhang, E. G., Smith, S. K., & Charnock-Jones, D. S. (2002). Expression of CD105 (endoglin) in arteriolar endothelial cells of human endometrium throughout the menstrual cycle. *Reproduction*, 124(5), 703-711.
67. Zhang, Y. E. (2008). Non-Smad pathways in TGF- $\beta$  signaling. *Cell research*, 19(1), 128-139.

**Table 1 – Clinical trials involving biological agents blocking endoglin/ALK1 signalling**

Target	Study drug	Company	Target disease	Trial phase	ClinicalTrials.gov identifier
endoglin	TRC105	Tracon Pharmaceuticals Inc	Metastatic breast cancer	I / II	NCT0132
			Advanced renal cell carcinoma	IB & II	NCT0180 NCT0172
			Recurrent glioblastoma multiforme	I / II, II & II	NCT0164 NCT0177 NCT0156
			Hepatocellular cancer	I / II & II	NCT0130 NCT0137
			Advanced urothelial carcinoma	II	NCT0132
			Advanced solid tumors	I	NCT0133
			Ovarian / peritoneal carcinoma	II	NCT0138
ALK1	PF-03446962	Pfizer Ltd	Advanced solid tumors	I	NCT0055
			Malignant pleural mesothelioma	II	NCT0148
			Advanced solid tumors	I	NCT0133
			Refractory urothelial cancer	II	NCT0162
			Recurrent liver cancer	II	NCT0191
BMP9	Dalantercept (ACE041)	Accelaron Pharma, Inc	Endometrial cancer	II	NCT0164
			Head & neck cancer	II	NCT0145
			Advanced renal cell carcinoma	II	NCT0172
			Ovarian / peritoneal carcinoma	II	NCT0172
			Refractory multiple myeloma	I	NCT0099

Source: <http://clinicaltrials.gov/>

