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REVIEW

Targeting cancer vasculature via endoglin/CD105: a novel antibody-based diagnostic and therapeutic strategy in solid tumours

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Endoglin/CD105 is well acknowledged as being the most reliable marker of proliferation of endothelial cells, and it is overexpressed on tumour neovasculature. Our current knowledge of its structure, physiological role, and tissue distribution suggests that targeting of endoglin/CD105 is a novel and powerful diagnostic and therapeutic strategy in human malignancies, through the imaging of tumour-associated angiogenesis and the inhibition of endothelial cell functions related to tumour angiogenesis. Among biotherapeutic agents, monoclonal antibodies have shown a major impact on the clinical course of human malignancies of different histotypes. Along this line, the potential efficacy of anti-endoglin/CD105 antibodies and their derivatives for clinical purposes in cancer is supported by a large body of available pre-clinical *in vitro* and *in vivo* data. In this review, the main findings supporting the translation of antibody-based endoglin/CD105 targeting from pre-clinical studies to clinical applications in human cancer are summarized and discussed.

Keywords Angiogenesis • Cancer • Endoglin/CD105 • Monoclonal antibodies • Therapy

1. Introduction

A large amount of experimental evidence has shown that endoglin/CD105 is expressed on endothelial cells of both mature and immature blood vessels,[1–3] and that it is overexpressed in vascular endothelial cells of tissues undergoing angiogenesis such as regenerating and inflamed tissues or tumours.[3–6] Furthermore, levels of endoglin/CD105 positively correlate with the extent of endothelial cell proliferation[4,5] and with the expression of proliferation markers in tumour endothelia.[6] In addition, endoglin/CD105 has been suggested to be the most suitable marker available to quantify tumour angiogenesis.[1,3,5,7,8] Lastly, intratumour microvessel density (IMVD) assessed by endoglin/CD105 staining strongly correlates with prognosis in cancer patients.[1,3,9,10] Altogether, these findings support the role of endoglin/CD105 as an optimal marker of proliferation of endothelial cells and its emerging clinical potential as prognostic, diagnostic, and therapeutic vascular target in human cancer.[1,4,11,12]

2. Structure

Endoglin/CD105 is a disulphide-linked homodimeric transmembrane protein[13–15] of 180 kDa[16,17] with short intracellular and transmembrane domains and a large extracellular region.[13–15] The latter consists of an orphan and a zona pellucida (ZP) domain, with an arginine-glycine-aspartic acid (RGD) binding motif,[15,18,19] while the intracellular region presents several potential phosphorylation sites.[20] Four potential N-linked glycosylation sites and a region of O-linked glycosylation rich in serine and threonine have been described.[15]

A high amino acid sequence homology was observed among human, porcine, and murine endoglin/CD105 proteins[21,22] with major differences in the extracellular domain.[22] The RGD tripeptide was detected only in the human protein. Furthermore, endoglin/CD105 shows homology with the TGF-β receptor (TβR) type III betaglycan in the transmembrane and cytoplasmic domains.[2,23]

The analysis of the three-dimensional structure of the extra-cellular region of endoglin/CD105 by single-particle electron microscopy identified three well-defined domains for each monomer region, including the two ZP regions and one orphan domain.[19]

The gene coding for endoglin/CD105 maps on human chromosome 9q34→qter[24] and cloning of its promoter demonstrated a strong and selective activity in endothelial cells.[25,26,27] Alternative spliced transcript variants encoding two different isoforms of endoglin/CD105 (L- and S-CD105) have been described.[28,29] The two

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forms differ in the length of the cytoplasmic domain and show a different pattern of tissue distribution.[27,28,29] L-CD105, the longer variant, is predominantly expressed in endothelial cells[29] and consists of 658 amino acids residues[15,21] with a higher extent of phosphorylation compared with S-CD105.[29]

3. Expression

Studies on the cellular and tissue distribution of endoglin/CD105 suggest its profound functional involvement in angiogenesis, vascular development, and homeostasis. Concerning the role of endoglin/CD105 in the development of the cardiovascular system, endoglin/CD105 was exclusively found on vascular endothelia of human embryos at 4–8 weeks of gestation, and it was transiently up-regulated during heart septation and heart valves formation.[30] Furthermore, an altered expression of endoglin/CD105 is observed in human foetuses with cardiac defects.[31] The involvement of endoglin/CD105 in vascular development is enforced by the observation that endoglin/CD105 *null* mice die during early gestation, due to structural alterations in the primitive vascular plexus of the yolk sac that prevents the formation of normal mature vessels, as reported for transforming growth factor (TGF)-β1, TGF-β receptor (TβR) type I activin receptor-like kinases (ALK)-1, and ALK-5 *null* mice.[32]

In human adult tissues, endoglin/CD105 expression is mainly restricted to vascular endothelial and stromal cells,[1,4] while it is weakly detectable on activated monocytes, macrophages, erythroid precursors, fibroblasts, mesangial cells, follicular dendritic cells, and melanocytes,[1,33] but it is highly expressed on syncytiotrophoblasts of term placenta.[34]

In cultured endothelial cells, the highest levels of endoglin/CD105 were detectable in cells showing levels of RNA, DNA, and proteins consistent with cellular proliferation and activation.[4] Consistently, levels of endoglin/CD105 expression correlated with the rate of cellular proliferation and density in cultures of human umbilical vein endothelial cells (HUVEC),[1,5] and high levels of endoglin/CD105 associated with other markers of proliferation such as cyclin A and Ki-67 in tumour endothelium.[6] Supporting these *in vitro* data, and highly suggestive for its involvement in tumour angiogenesis, an up-regulated expression of endoglin/CD105 was found on endothelia of angiogenetic blood vessels within tumour tissues[4,5,7,8] where, at variance with the pan-endothelial markers CD31 and CD34, it was also detected in neovessels with strong remodelling activity and in immature neovessels.[3,35] Among tumours of different histotype, staining of endoglin/CD105 was invariably observed in peri- and intratumoural blood vessels and on tumour stromal components;[1,5,36] and, on the other hand, only a weak expression of endoglin/CD105 was detected in the cytoplasm of neoplastic cells of selected histotypes.[1,5,36]

4. Functions

Biochemical and biological functions of endoglin/CD105 are under active investigation, and new data continue to emerge on its functional role within the TGF-β receptor complex, on its modulation of cellular responses to TGF-β, and on its involvement in vascular physiology and angiogenesis.[12,14]

As reported for betaglycan, endoglin/CD105 is a TβR type III auxiliary receptor[12,16,37] that modulates the signalling response to TGF-β, a pleiotropic cytokine involved in tumour development and angiogenesis through the regulation of cellular functions including proliferation, differentiation, and migration.[14,37] Endoglin/CD105 binds different components of the TGF-β superfamily such as activin-A, bone morphogenetic protein (BMP)-7, and BMP-2.[17] In particular, endoglin/CD105 binds TGF-β1 and TGF-β3 with high affinity but, at difference with betaglycan, it does not bind TGF-β2[16,17,23] and only a fraction of endoglin/CD105 expressed on endothelial cells binds TGF-β.[16]

It has been demonstrated that endoglin/CD105 requires the association with TβR type II to bind ligands,[17,23,38] and that it can interact with TβR type I or type II in the absence of ligand.[38] Based on different experimental data, it has been proposed that through its interactions with the TβR type I and type II, endoglin/CD105 regulates their phosphorylation status and subsequently their signalling ability.[38] However, endoglin/CD105 can also act independently of the TGF-β signalling pathway.[12]

In endothelial cells, two TβR type I pathways with opposite effects have been identified: the ALK-5 that induces Smad 2/3 phosphorylation and the ALK-1 that induces Smad 1/5 phosphorylation.[39,40] The latter is known to promote endothelial cell proliferation, migration, and tube formation, while the first inhibits these cellular responses to TGF-β.[40] Evidence suggests that endoglin/CD105 is required for TGF-β/ALK1 signalling and indirectly inhibits TGF-β/ALK5 signalling.[40,41]

Down-regulation of endoglin/CD105 expression through small interfering RNA demonstrated that endoglin/CD105 promotes TGF-β-induced Smad 1/5 phosphorylation, and proliferation and migration of murine endothelial cells via ALK-1 receptor. In fact, it has been shown that loss of endoglin/CD105 abrogates ALK-1 signalling and endothelial cells proliferation, while endoglin/CD105 haploinsufficiency leads to a down-regulation of surface ALK-5 expression, probably as an adaptation mechanism.[39] These results suggest that endoglin/CD105 is required for efficient TGF-β/ALK-1 signalling and that it acts as a modulator factor of the balance between TGF-β/ALK-1 and TGF-β/ALK-5 signalling pathways[39] (*Figure 1*).

Available data on the interaction of endoglin/CD105 with TβR type I and type II suggest that the extracellular and the cytosolic domains of endoglin/CD105 play a distinct role in the TGF-β receptor signalling,[14,17,23,38] and that the association of CD105 with ALK-1 is crucial for the response of endothelial cells to TGF-β.[39,42,43] Along this line, distinct levels of endoglin/CD105 differentially modulate cellular responses to TGF-β including: cellular proliferation, adhesion and migration, homotypic cell aggregation, and expression of selected matrix components (i.e. plasminogen activator inhibitor-1, collagen, fibronectin, lumican).[1,23,37,44–48] In particular, the deletion of endoglin/CD105 enhances the ability of TGF-β1 to suppress growth, migration, or microvessels formation in HUVEC and smooth muscle cells, as well as apoptosis induced by hypoxia or TGF-β1 in endothelial cells.[37,45,49] Consistently, reduced proliferation and migration, increased basal or TGF-β1-mediated collagen synthesis, impaired capillary tube formation, and vascular endothelial growth factor (VEGF) secretion are reported in endothelial cells derived from *endoglin/CD105* $\pm$ adult mice.[50]

Supporting its role in vascular homeostasis and integrity, endoglin/CD105 was identified as a component of endothelial nitric oxide synthase (eNOS) activation pathway and a modulator of COX-2 expression and activity.[51–54] Thus, endoglin/CD105 can also regulate the vascular tone by maintaining the fine balance between eNOS and COX-2 in endothelial cells.[54] Mutations in the *endoglin/CD105* gene

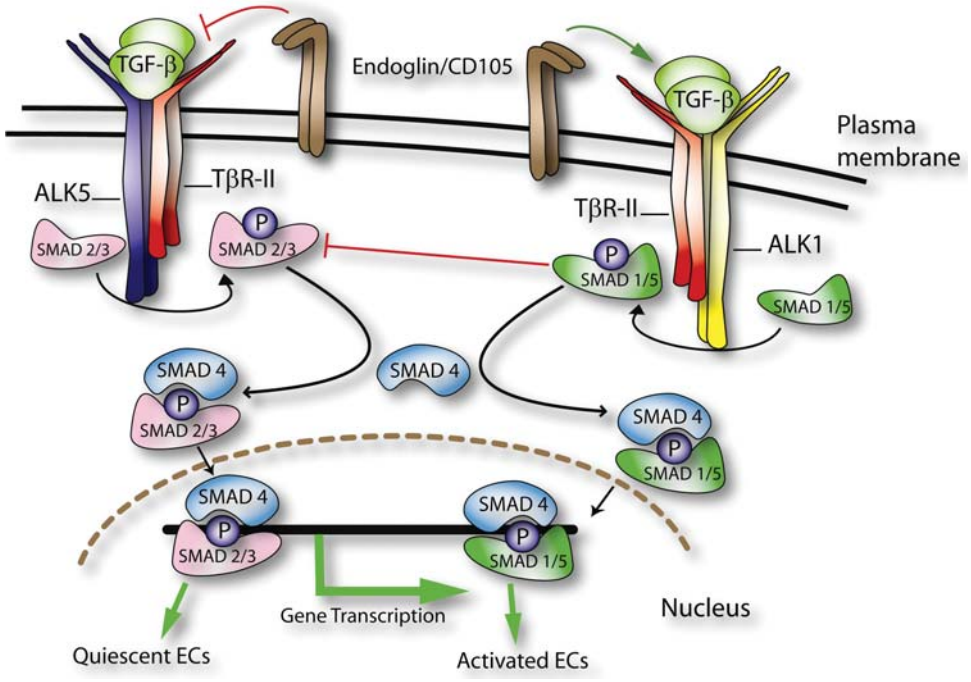

Figure 1 A schematic hypothetical role of endoglin/CD105 in TGF-β/ALK-1 and TGF-β/ALK-5 signalling pathways in endothelial cells. Available data in literature suggest that the levels of expression of endoglin/CD105 on endothelial cells (ECs) can affect the response of endothelial cells to TGF-β modulating their proliferation. In endothelial cells, TGF-β can activate two type I receptor pathways with opposite effects: ALK-5 inducing Smad 2/3 phosphorylation and ALK-1 promoting Smad 1/5 phosphorylation. Endoglin/CD105 binds TGF-β by associating with TGF-β signalling receptors type (TβR)-II. This association results in an altered phosphorylation state of TβR-II promoting endothelial cells proliferation via TGF-β/ALK-1 signalling pathway and inducing an indirect inhibition of ALK-5 signalling pathway. Upon activation, phosphorylated Smads form heteromeric complexes with the common mediator Smad 4, which in the nucleus act as transcription factor complexes regulating the transcriptional activity of target genes.

are associated to hereditary hemorrhagic telangiectasia (HHT) type 1,[19,55,56] an hereditary disease characterized by arteriovenous malformations and recurrent bleeding. It was in fact reported that endoglin/CD105 haploinsufficiency is the cause of defective vascular repair in HHT type 1 that may in part explain the heterogeneity of symptoms observed among members of the same family.[57]

Inhibition of endothelial cell apoptosis is crucial during angiogenesis and vasculogenesis, and it is well-known that pro-angiogenetic factors also promote survival of endothelial cells. In this setting, endoglin/CD105 was shown to protect hypoxic endothelial cells from apoptosis either in the presence or absence of TGF-β1.[49] Supporting this finding, an inverse correlation between the extent of apoptosis and IMVD assessed by anti- endoglin/CD105 antibodies was reported in non-small cell lung cancer patients.[58] Thus, in tumour angiogenesis, endoglin/CD105 may also play a role in activating anti-apoptotic signals in hypoxic endothelial cells.

At variance with the extensive knowledge on the activity of endoglin/CD105 in endothelial cells, limited and conflicting data are available on the functional role of endoglin/CD105 on neoplastic cells. *In vitro* studies reported that overexpression of endoglin/CD105 on metastatic prostate cancer[59,60] and oesophageal squamous carcinoma cells[61] decreased their invasiveness, while this ability was improved by the up-regulation of endoglin/CD105 in breast cancer cells.[62] These findings, and its overall limited expression on neoplastic cells,[1,5,36] clearly suggest that additional studies are required to unveil the possible functional significance of endoglin/CD105 on tumour cells of different histotype.

5. Tumour angiogenesis

A number of studies have reported that the assesment of IMVD by endoglin/CD105 staining represents a good prognostic indicator in different malignancies. Endoglin/CD105-positive blood vessels count negatively correlated with overall survival, disease-free survival, or presence of distant metastases in a wide range of human tumours.[9,10,12,36,63,64] Additionally, IMVD score by anti-endoglin/CD105 mAb was shown to positively correlate with Gleason score in prostate cancer patients[3] and with the tumour stage in squamous cell carcinomas of the oral cavity.[65] These studies also indicated that blood vessels count by endoglin/CD105 staining is a more informative marker of prognosis as compared with staining by other pan-endothelial markers.[1,9,10,12] Consistently, an increase in IMVD was assessed by endoglin/CD105 mAb during progressive stages of colorectal carcinogenesis from low- to high-grade dysplasia, and from high-grade dysplasia to carcinoma.[66] This evidence provided indirect support to the role of endoglin/CD105 in tumour angiogenesis and to the usefulness of endoglin/CD105 targeting for anti-angiogenetic therapy of cancer.[1,12]

To specifically investigate the role of endoglin/CD105 in tumour angiogenesis and growth, endoglin/CD105 haploinsufficient

(*endoglin/CD105*+/−) and control littermates (*endoglin/CD105*+/+) mice were subcutaneously inoculated with Lewis lung carcinoma cells. The results of these studies indicated that endoglin/CD105 deficiency decreased tumour vascularization and growth in mice, further supporting the idea that endoglin/CD105 is involved in tumour angiogenesis.[67] In addition, a significant decrease in the levels of eNOS and of phosphorylated eNOS was reported in *endoglin/CD105*+/− mice compared with control mice, suggesting that this deficiency might be responsible of the decreased angiogenesis-dependent tumour growth in *endoglin/CD105*+/− mice.[67] Instead, the lower tumour vascularization in *endoglin/CD105*+/− mice was not associated with lower levels of the major angiogenetic factors, such as hypoxia inducible factors and VEGF, advising that the involvement of endoglin/CD105 in tumour angiogenesis occurs downstream of these factors.[67]

Unexpectedly, an increased number of *endoglin/CD105*-positive vessels and an up-regulation of *endoglin/CD105* gene expression within tumours were reported in mice following anti-angiogenetic treatment with anti-VEGF mAb[68] or with anti-VEGF Receptor-2[69] mAb, respectively, likely as a consequence of increased hypoxia induced by the treatment.

6. Targeting of endoglin/CD105 for cancer imaging

Solid malignancies are generally highly vascularized, and endothelial cells lining the tumour vasculature proliferate much faster than endothelial cells of blood vessels in normal tissues.[70,71] Thus, an ideal target for the imaging of tumour vasculature should be over-expressed in actively proliferating endothelial cells but it should be weakly expressed or undetectable in quiescent endothelial cells; additionally, it should be expressed in a large amount on the luminal surface of the blood vessels. Based on these notions, the evidence that levels of endoglin/CD105 expression correlate with the proliferation rate of endothelial cells,[4–6] and that it is overexpressed in endothelia within neoplastic tissues compared with those of normal tissues, prompted investigations on endoglin/CD105 as a potential target for the diagnostic imaging of solid tumours.[5,72] The feasibility of endoglin/CD105 targeting for diagnostic applications was addressed in two distinct animal models and utilizing two different radio-labelled anti-endoglin/CD105 mAb. The results indicated that targeting of endoglin/CD105 is a useful and safe procedure for tumour imaging,[5,72] regardless of tumour histotype and independently from endoglin/CD105 expression on neoplastic cells.[5] In particular, the intravenous injection of the ^{125}I-labelled anti-endoglin/CD105 mAb MAEND3 efficiently imaged spontaneous mammary canine adenocarcinomas. The up-take of the mAb into the tumour areas was described as rapid and intense, without systemic side effects in injected animals during a 3-month follow-up after imaging procedures.[5] Consistent with these initial findings, the intravenous administration of the ^{111}In-labelled anti-endoglin/CD105 mAb MJ7/18 effectively imaged allografts of melanoma in C57BL/6 mice.[72] The autoradiography and the subsequent immunohistology showed that the highest levels of mAb were concentrated in the periphery of the tumour mass where vessel density is prominent, with a heterogeneous distribution in the tumour centre. The blood half-life of the antibody was reported to be less than 1 min.[72]

Important clinical implications of endoglin/CD105 targeting in improving cancer diagnosis were further shown by an *ex vivo* study in human renal carcinoma.[73] The ^{99}Tcm-labelled anti-endoglin/CD105 mAb E9 was perfused in the renal artery of freshly excised kidneys from seven patients diagnosed with renal carcinoma. The scintigraphy identified hot spots of radioactivity, which matched with the position of the neoplastic lesions. The specificity of the localization of the labelled anti-endoglin/CD105 mAb into the tumour mass was confirmed by the observation that a previous perfusion of unlabelled mAb completely blocked the localization of ^{99}Tcm-conjugated mAb. Noteworthy, the anti-endoglin/CD105 mAb identified two tumour masses previously undetected by pre-surgery magnetic resonance imaging scan.[73] Altogether, these imaging studies clearly indicate that *in vivo* anti-endoglin/CD105 mAb specifically and strongly target the tumour vasculature, and provide support to their usefulness also for therapeutic anti-angiogenic approaches in human cancer.

Targeting of endoglin/CD105 has also been suggested as a possible strategy to monitor the response to cancer therapy. Microbubbles are suitable contrast agents for the enhancement of ultrasound images, and avidin can be incorporated into the shell of microbubbles, acting as a direct ligand for biotinylated mAb. Utilizing this approach, microbubbles conjugated with the anti-endoglin/CD105 mAb MJ7/18 specifically bound *in vitro* to murine brain capillary endothelial cells expressing high levels of endoglin/CD105, but not to murine fibroblasts expressing low levels of endoglin/CD105.[74] Being ultrasound a powerful non-invasive diagnostic tool, microbubbles conjugated to mAb MJ7/18 were also utilized to image and quantify vascular effects of cytotoxic therapy in a mouse model of pancreatic adenocarcinoma.[75] The results obtained showed a significantly decreased ultrasound signal in tumours treated with gemcitabine.[75] Thus, microbubbles conjugated with anti-endoglin/CD105 mAb may represent a foreseeable non-invasive tool for the imaging of tumour angiogenesis and for monitoring vascular effects of anti-cancer therapy.

7. Targeting of endoglin/CD105 for cancer treatment

The well-acknowledged notion that angiogenesis supports primary tumour growth and its metastatic progression has prompted the investigation of different anti-cancer strategies aimed to inhibit the formation of new blood vessels and/or to disrupt tumour-associated blood vessels.[76,77] In principle, these therapeutic modalities bear the advantage of overcoming several limitations commonly related to the targeting of transformed cells such as their inherent drug resistance, the poor delivery of drugs to neoplastic cells, and the need to target a biologically highly heterogeneous malignant cell population.[76,78]

Among antibodies with anti-angiogenetic activity so far tested in cancer patients, only the humanized anti-VEGF mAb bevacizumab has received approval by Regulatory Agencies, though for selected clinical indications.[79–81] Thus, different novel agents are currently under active clinical investigation for cancer treatment, with the intent to starve neoplastic cells by blocking their tumour blood supply. In this setting, targeting of proliferating endothelial cells is generally considered the most promising therapeutic strategy to control tumour angiogenesis.

Strong experimental support to the possible use of anti-endoglin/CD105 mAb for therapeutic vascular targeting in cancer derives from *in vitro* studies demonstrating that different anti-endoglin/CD105 antibodies inhibit proliferation, migration and adhesion of endothelial cells[33,82,83] and/or induce apoptosis in human umbilical vein endothelial cells.[67] Moreover, selected anti-endoglin/CD105 mAb showed a synergistic activity with TGF-β in the inhibition of endothelial cells proliferation.[82]

Diverse engineered antibodies directed to endoglin/CD105 have also been generated and utilized to target or to deliver pharmacological agents to endothelial cells *in vitro*. A bispecific antibody directed to endoglin/CD105 and CD3 was utilized to mediate killing of endoglin/CD105-positive endothelial cells by cytotoxic T lymphocytes.[84] Single-chain Fv fragments directed to endoglin/CD105 were used to generate immunoliposome of encapsulated therapeutic drugs to target endothelial cells;[85] these complexes were able to internalize into and kill endothelial cells.[85] Nanobodies against endoglin/CD105 have also been recently described[86] and represent another promising tool for therapeutic applications of endoglin/CD105 since they are small, non-immunogenic, stable, highly soluble, and easy to produce.

The therapeutic potential of endoglin/CD105 targeting has been already extensively investigated at pre-clinical level also *in vivo*. Several studies demonstrated a long-lasting regression/suppression of tumour growth and metastasis in SCID mice by the immunotoxin-conjugated,[87,88] radiolabelled,[89] or naked anti-endoglin/CD105 mAb[90–93] termed SN6f, SN6j, and SN6 k, likely mediated by the inhibition of tumour-associated angiogenesis, and/or by the destruction of tumour-associated vasculature. Furthermore, utilizing a human skin/SCID mouse chimera model, the naked human anti-endoglin/CD105 mAb SN6f, SN6j, and SN6 k suppressed human blood vessels but poorly inhibited murine vessels in established tumours.[92] Interestingly, this study also showed that the *in vivo* anti-tumour efficacy of tested anti-endoglin/CD105 mAb was not proportional to their antigen-binding avidities, and that the combined administration of SN6f and SN6 k, directed to non-overlapping epitopes of endoglin/CD105, showed an additive anti-tumour effect.[92] Furthermore, the co-administration of mAb SN6j and cyclophosphamide had a synergistic anti-tumour efficacy and completely suppressed tumours in some chimeras.[92]

Growth suppression of established tumours of colon-26 murine colon carcinoma cells and of 4T1 murine mammary carcinoma cells by systemic administration of unconjugated anti-endoglin/CD105 mAb SN6j was also reported in immunocompetent tumour bearing BALB/c mice.[91] Differences in tumour growth rate and therapeutic response were found to depend on the tumour location both in immunocompetent BALB/c mice and in immunodeficient SCID mice.[91] The anti-endoglin/CD105 mAb was more effective against skin tumours with a low growth rate, as compared with rapidly growing intramuscular tumours.[91] Consistent with these studies, the anti-tumour activity of a recently characterized anti-endoglin/CD105 mAb was also demonstrated in BALB/c and C57BL mice inoculated with H22 or Hepa1–6 hepatoma cells, respectively.[94] In both models, the administration of the mAb induced a significant inhibition of tumour growth and increased the survival rate.[94] The analysis of the tumour tissues excised from treated mice showed an increase in apoptotic cells and a decrease in microvessels, compared with those removed from control mice.[94]

The anti-tumour activity of anti-endoglin/CD105 mAb was found improved in immunocompetent compared with immunodeficient mice.[90] Confirming the hypothesis that T cell immunity could play a role in enhancing the efficacy of anti-endoglin/CD105 antibody-based therapy, the administration of an immune activator CpG ODN, enhanced the anti-tumour efficacy of the anti-endoglin/CD105 mAb SN6j in immunocompetent BALB/c mice, but not in immunodeficient SCID mice. Supporting the notion that immune mechanisms are involved in antibody-based endoglin/CD105-targeted tumour therapy, the anti-tumour efficacy of the SN6j mAb was abrogated when CD4+ and/or CD8+ T cells were depleted from BALB/c mice.[90]

Noteworthy for perspective clinical applications in patients, three mAb (SN6a, SN6j, and SN6 k) recognizing distinct epitopes of endoglin/CD105 showed therapeutic efficacy against tumour metastases in BALB/c mice inoculated with 4T1 murine mammary carcinoma or colon-26 murine adenocarcinoma cell lines.[93]

In order to assess the potential clinical application of anti-endoglin/CD105 antibodies, the recombinant human/mouse chimeric antibody of IgG1 isotype designated c-SN6j was generated from mAb SN6j, based on its strong anti-tumour efficacy *in vivo* and its strong ability to inhibit the proliferation of endothelial cells *in vitro*.[95] When administered in monkeys, mAb c-SN6j showed pharmacokinetic parameters comparable to those reported in humans for different therapeutic mAb.[95]

In an attempt to construct a novel anti-endoglin/CD105 immunoconjugated mAb with limited unspecific toxicity, the type 2 ribosome-inactivating protein nigrin b was linked to the anti-human endoglin/CD105 mAb 44G4. This 44G4 nigrin b immunotoxin specifically killed murine fibroblasts expressing the short isoform of human endoglin/CD105 and in a perinuclear region.[96]

A phase I study, first-in human, with the human/murine chimeric anti-endoglin/CD105 mAb TRC105 in patients with refractory advanced or metastatic solid cancer is ongoing. Objectives of the study are to evaluate safety and tolerability of escalating doses of the therapeutic mAb, its pharmacokinetics and immunogenicity, as well as signs of clinical activity. Preliminary data were most recently reported showing that treatment is well tolerated at doses up to 1 mg/Kg and with evidence of clinical activity. Two dose-limiting toxicity were observed: a Grade 3 hypersensitivity reaction and a Grade 4 bleeding. Though initial, these findings indicate that anti-VEGF mAb and anti-endoglin/CD105-based treatment might share bleeding as a common adverse event.[97]

8. Conclusions and future directions

In light of its involvement in vascular development, morphogenesis and physiology, and of its strong expression on blood vessels of tumour tissues, the potential of endoglin/CD105 as vascular target for diagnostic and therapeutic anti-angiogenetic strategies in cancer has been extensively investigated at pre-clinical level both *in vitro* and *in vivo* (summarized in *Table 1*).

Endoglin/CD105 is overexpressed on proliferating endothelial cells[1–5] and in the vascular endothelium within tumour tissues,[1,4,6–8,98] but also in endothelia of normal tissues.[98,99] This latter notion raised concerns about potential side effects of clinical applications of endoglin/CD105 targeting in cancer patients.[98–102] However, it was convincingly argued that most tissue antigens targeted by therapeutic antibodies that are successfully employed in the clinic are not tumour specific.[100] Nevertheless, not all available anti-endoglin/CD105 mAb are likely good candidates for therapeutic applications

Table 1 Pre-clinical studies with different anti-endoglin/CD105 mAb

mAb	*In vitro*		*In vivo*		
	Activity	**Cell type**	**Activity**	**Tumour type**	**Host**
MJ7/18	Target binding[74]	EC (M)	Cancer imaging[72] Vascular imaging[75]	Melanoma (M) Pancreatic adenocarcinoma (M)	C57BL/6 mice *nu/nu* mice
MAEND3	–		Cancer imaging[5]	Spontaneous mammary carcinoma	Beagle dog, mixed breed dog
E9	–		Cancer imaging[73]	Renal carcinoma (H)	Excised human kidneys
SN6f	–		Anti-tumour[87,89,92] Anti-angiogenetic[87,89,92]	Breast cancer (H)	H skin/SCID mouse chimeras, SCID mice
SN6k	–		Anti-tumour[88,92,93] Anti-angiogenetic[88,92]	Mammary carcinoma (M), Colon adenocarcinoma (M), Breast cancer (H)	BALB/c mice, H skin/SCID mouse chimeras, SCID mice
SN6J	Inhibition of proliferation[82,90] Induction of apoptosis[90] ADCC[93]	HUVEC	Anti-tumour[90–93] Anti-angiogenetic[92,93]	Mammary carcinoma (M), Colon adenocarcinoma (M), Breast cancer (H)	BALB/c mice, H skin/SCID mouse chimeras, SCID mice
SN6	Inhibition of proliferation[82]	HUVEC	–		
SN6a	Inhibition of proliferation[82]	HUVEC	Anti-tumour[93] Anti-angiogenetic[93]	Mammary carcinoma (M), Colon adenocarcinoma (M)	BALB/c mice
SN6h	Inhibition of proliferation[82]	HUVEC	–		
TEC11	Inhibition of proliferation[4,83] Inhibition of urokinase production[83]	HUVEC, HDMC, vascular EC (H)	–		
44G4	Target binding[96]	Fibroblasts (M)	–		

EC, endothelial cells; HUVEC, human umbilical vein endothelial cells; ADCC, antibody-dependent cellular cytotoxicity, HDMC, human dermal microvascular endothelial cells; H, human; M, murine.

Table 2 Points to consider about anti-endoglin/CD105 mAb use for imaging and treatment of human tumours

Strenghts	Limitations
Optimal accessibility from the blood stream	Presence of a soluble form
Over-expression in tumour endothelia compared with endothelia of normal tissues	Extent of tumour vascularization
Stable expression	Limited by tumour size? (imaging)
Limited background (imaging)	Presence and extent of tumour necrosis (imaging)
No tumour-histotype specificity	Bleeding as side effect? (therapy)
Independence from the expression on neoplastic cells	
Detectable in mature and immature neovessels	
No major side effects in animal models	
Useful to evaluate the efficacy of anti-angiogenetic treatments	

in cancer since differences in their reactivity with the tumour vasculature have been observed.[92,98,100]

As a whole, the pre-clinical data available undoubtedly point to endoglin/CD105 as an attractive vascular target to design novel antibody-based diagnostic and therapeutic strategies shared by different human malignancies, suggesting that times are mature to identify the most appropriate therapeutic applications of endoglin/CD105 targeting in the clinical setting of cancer.[103] Along this line, it has been recently shown that, unlike other pan-endothelial markers, endoglin/CD105 is overexpressed in hepatic epithelioid hemangioendothelioma (HEH), a rare neoplasm of endothelial origin, clearly pointing to endoglin/CD105 as a possible candidate for the therapeutic targeting of HEH.[104]

It is finally worth mentioning that, other than on endothelial cells, endoglin/CD105 is weakly expressed on selected cell types that indirectly contribute to tumour angiogenesis such as mural, stromal, and inflammatory cells.[1] Thus, it seems reasonable to envisage that the anti-angiogenetic efficacy of antibodies directed to endoglin/CD105 might be strengthened by their ability to target multiple cell types involved in the angiogenetic process besides endothelial cells.

In the last few years significant advances have been made on the biology of endoglin/CD105 in cancer, allowing to identify potential advantages and drawbacks associated with its diagnostic and therapeutic use (*Table 2*); altogether, time seems mature to translate the results emerged from pre-clinical research to the clinical setting. Along this line, a multicenter clinical trial is in progress[97] and its results are eagerly awaited to validate endoglin/CD105 as a novel target for cancer treatment.

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