MONOCLONAL ANTIBODIES AGAINST METZINCIN TARGETS

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Abbreviations: Cat, catalytic domain; C_H, C_L, constant domains of antibody heavy and light chains, respectively; CysR, Cys-Rich domain; Fc, crystallisable fragment; Hpx, hemopexin-like domain; IgG, immunoglobulin G; mAb, monoclonal antibody; MMP, matrix metalloproteinases; MT-MMP, membrane-type MMP; OA, osteoarthritis; RA, rheumatoid arthritis; scFv, single chain variable fragment; Sp, spacer domain; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor; TS, thrombospondin-like motif; TTP, thrombotic thrombocytopenic purpura; UC, ulcerative colitis; V_H, variable region of the heavy chain; V_L, variable region of the light chain; VWF, von Willebrand Factor.

Abstract: The metzincin clan of metalloproteinases includes the MMP, ADAM and ADAMTS families, which cleave extracellular targets in a wide range of (patho)physiological processes. Antibodies constitute a powerful tool to modulate the activity of these enzymes for both therapeutic and research purposes. In this review, we give an overview of monoclonal antibodies (mAbs) that have been tested in preclinical disease models, human trials and important studies of metzincin structure and function. Initial attempts to develop therapeutic small molecule inhibitors against MMPs were hampered by structural similarities between metzincin active sites and, consequently, off-target effects. Therefore, more recently, mAbs have been developed that do not bind to the active site but bind to surface-exposed loops that are poorly conserved in closely related family members. Inhibition of protease activity by these mAbs occurs through a variety of mechanisms, including 1) barring access to the active site 2) disruption of exosite binding and 3) prevention of protease activation. These different modes of inhibition are discussed in the context of the antibodies' potency, selectivity and, importantly, the effects in models of disease and clinical trials. In addition, various innovative strategies that were used to generate anti-metzincin mAbs are discussed.

Keywords: ADAMS, ADAMTS, MMP, metzincin, monoclonal antibodies, exosites

1. Introduction

Monoclonal antibodies (mAbs) are widely used as drugs and in research. The isotype used is usually immunoglobulin G (IgG). IgGs are tetramers of ~150 kDa, which comprise a pair of identical heavy and light chains linked by disulfide bonds (Fig 1). IgGs are divalent, i.e they contain two antigen binding sites, each composed by the variable regions of the heavy and light chains (V_H and V_L, respectively). Increasingly, smaller antibody fragments that retain the antigen binding site are used. scFv (*s*ingle *c*hain *v*ariable *F*ragment) comprises the variable regions of the V_H and V_L of an IgG molecule connected with a short glycine-rich linker. This fusion protein retains the specificity of IgG and constitutes a minimal antigen binding site. Fabs (*a*ntigen-*b*inding *F*ragments) are composed of one constant and one variable domain from the heavy and light chains connected by a disulfide bond. scFv can also be expressed as fusion proteins together with the Fc (*c*rystallizable *F*ragment) region of the IgG molecule, which comprises the constant domains (C_H) of the heavy chains. The resulting molecule (scFv-Fc),

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retains both the antigen binding site, provided by the scFv, and the immune effector functions, provided by the Fc region. The Fc region is recognized by Fc receptors on various immune cells and mediates cytotoxic functions and clearance of immune complexes of mAbs bound to their extracellular target. The combination of high potency and high selectivity for their target has not only made mAbs an important new class of therapeutics, but also an invaluable tool in studies of protein function, including that of the metalloproteinase clan called metzincins.

Metzincins are metallopeptidases that share a highly similar catalytic site. This contains three histidines that coordinate a zinc ion and a glutamate, which together with a bound water molecule drive the hydrolysis of the substrate peptide bond. The structure of their catalytic site is further formed by the consensus sequence HEXXHXXG/NXXH/D. This motif is followed C-terminally by a conserved methionine residue which constitutes a tight turn (named "Met-turn") and contributes to the structural integrity of the catalytic domain (Bode et al., 1993; Tallant et al., 2010). The best known metzincin families are the matrix metalloproteinases (MMPs) and the adamalysins, which are sometimes called reprolysins and include ADAM and ADAMTS subfamilies. Other metzincin clan members are the astacins, serralysins, snapalysins, leishmanolysins, pappalysins, archaemetzincins and fragilysins (Gomis-Rüth et al., 2012). In this review we give an overview of promising mAbs that have been used in human trials, preclinical models and important studies of the structure and function of metzincins.

2. MMPs

Matrix metalloproteinases (MMPs or matrixins) play key roles in remodelling and turnover of extracellular matrix and are involved in embryonic development, wound healing, angiogenesis, arthritis, cardiovascular diseases, and cancer (Murphy and Nagase, 2008). They also cleave intracellular substrates (Cauwe and Opdenakker, 2010; Ali et al., 2010; Jobin et al., 2017) and act as transcriptional activators (Marchant et al., 2014).

The MMP family comprises 23 members, which are generally classified as collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10), matrilysins (MMP-7 -11, and -26) and membrane-type (MT)-MMPs (MMP-14, -15, -16, -17, -24 and -25). They consist of a prodomain which maintains the zymogen form, a zinc and calcium-dependent catalytic domain (Cat), a linker peptide (hinge region), and a hemopexin-like (Hpx) domain (Fig 2). Remarkably, the Cat domain of MMP-2 and -9 have fibronectin II-like repeats inserted into a central loop region of the Cat domain, while maintaining the same general fold of the metzincin Cat-domain.

Small molecule inhibitors have been developed that target the catalytic zinc ion of MMPs, but their selectivity was generally poor, which can be explained by the similarity of the active sites of MMPs and metzincins in general (Coussens et al., 2002). MAbs, however, have the advantage that they can be developed to bind variable regions outside the catalytic site, while maintaining inhibitory potential by blocking access to the active site or exosites (Table 1).

2.1. Gelatinases (MMP-2 and -9)

The gelatinase subfamily consists of MMP-2 and MMP-9. MMP-9 cleaves collagen IV and laminin in the basement membrane, which is thought to affect tumour cell invasion and metastasis (Overall and Kleifeld, 2006). Matrix remodelling by gelatinases also appears to promote the growth of tumour cells, probably by facilitating vascularization (Hu et al., 2007). MMP-9 has also been implicated in inflammation-related tissue damage. In this context, it has been studied in inflammatory bowel disease, cystic fibrosis, rheumatoid arthritis (RA), and

bullous pemphigoid (Hu et al., 2007; Gaggar et al., 2007; Fanjul-Fernandez et al., 2010; de Bruyn et al., 2016). MMP-9 is, therefore, considered a drug target in several of these conditions. One of the first inhibitory mAbs that was developed against MMPs in the early 1990's, (REGA-3G12), binds the Cat domain of MMP-9 (Paemen et al., 1995; Martens et al., 2007). It proved very useful a few years later when it was used to elucidate a physiological role of MMP-9 in haematopoiesis (Pruijt et al., 1999). Later on, it also provided important evidence that suggested a potential benefit of targeting MMP-9 in bullous pemphigoid (Shimanovich et al., 2004). Interestingly, another early anti-MMP-9 mAb (Höyhtya et al., 1990) increased proteolysis of type IV collagen instead of inhibiting it, but unfortunately the mechanism was not elucidated.

More recently, Sela-Passwell et al. (2011) immunized mice with a synthetic molecule (a Tris-imidazole-zinc complex) that mimics the structure of the zinc-histidine complex present in the active site of metzincins. They then isolated two mAbs (SDS3 and SDS4) that specifically inhibit MMP-2 and MMP-9, but showed no or minimal inhibition of other MMPs and did not bind to free zinc-ions. However, this strategy does not allow prediction of the metzincin target before immunization and the risk of cross reactivity with other metalloproteinases is high. The crystal structure of the SDS3 Fab-MMP-9 Cat domain complex confirmed that the antibody directly bound to the active site zinc through the hydroxyl group of a tyrosine residue in its antigen binding site. Administration of either SDS3 or SDS4 in mouse models of ulcerative colitis (UC) attenuated the severity of the disease, suggesting that targeting of MMP-2 and MMP-9 may be a viable therapeutic option in UC.

An anti-MMP-9 mAb developed by Gilead Sciences (GS-5745) recognizes with much higher affinity the proform rather than the active enzyme and inhibits MMP-9 activation. In addition, it inhibits MMP-9 catalytic activity directly by ~50%, probably because it sterically hinders access to the active site (Appleby, et al., 2017). It was efficacious in preclinical models of UC and cancer (Marshall et al., 2015) and a phase I randomized clinical trial showed it did not induce musculoskeletal syndrome (Sandborn et al., 2016), a side-effect frequently associated with broad spectrum small molecule MMP inhibitors (Coussens et al., 2002). GS-5745 is currently being tested in several clinical trials under the commercial name of Andecaliximab , including a phase 3 trial in patients with gastric cancer (ClinicalTrials gov Identifier NCT02545504) and phase II trials in patients with Crohn's disease (NCT02405442, now completed), RA (NCT02862574) and cystic fibrosis (NCT02759562). A combined phase 2/3 trial in patients with UC (NCT02520284) was stopped due to lack of efficacy.

2.2. MMP-14

MMP-14 (MT1-MMP) can digest several extracellular matrix proteins (collagens, fibronectin, and laminin) but one of its important roles is the initiation of proteolytic cascades through the activation of other proMMPs such as proMMP-2 (Ohuchi et al., 1997, Sato et al., 1994). The initial activation of proMMP-2 requires the formation of a complex between proMMP-2, Tissue Inhibitor of Metalloproteinase-2 (TIMP-2), and an MMP-14 homo-dimer. TIMP-2 inhibits one of the MMP-14 molecules in the homodimer through its N-terminal domain whereas its C-terminal domain binds to the Hpx domain in proMMP-2. This allows activation of proMMP-2 by the uninhibited MMP-14 molecule in the homodimer and subsequent further autocatalytic processing to the fully active form (Itoh, 2015).

Inhibition of MMP-14 has attracted considerable interest due to its involvement in several pathophysiological processes, including cancer cell invasion, metastasis, angiogenesis (Itoh, 2015), RA (Miller et al., 2009) and lung infection (Talmi-Frank et al., 2016). The first mAb selectively targeting MMP-14 was DX-2400, developed by Dyax Corp (Devy et al., 2009). It was selected from a human Fab phage display library against the Cat domain of MMP-14 and directly competes with a synthetic peptide substrate. It was shown to be effective in *in vitro* and *in vivo* models of angiogenesis, tumour growth and metastasis (Devy et al., 2009; Ager et al., 2015). Moreover, it significantly reduced cartilage degradation and disease progression in a mouse model of RA, where it exerted its effect mainly by reducing the spread of the disease to unaffected joints, with minimal effect on swelling and bone erosion (Kaneko et al., 2016). This study showed the importance of MMP-14 in cartilage degradation and migration of rheumatoid synovial fibroblasts to unaffected joints. Importantly, the combined inhibition of MMP-14 and tumour necrosis factor (TNF) synergistically inhibited both joint damage and synovial inflammation (Kaneko et al., 2016).

Raising conventional antibodies that bind the active site of metzincins is complicated by the fact that the active site is often buried in a cleft that is poorly accessible by the antibodies due to the overall shape of their antigen binding surface (cave-like, grooved or flat) (Lauwereys et al., 1998). To address this, Nam et al (2016) introduced the longer camelid complementarity determining region (CDR)-H3 into the human antibody scaffold. The idea is that the long, convex-shaped, camelid-like paratopes are better able to insert into the active site cleft than conventional antibodies. A human Fab library was then made to isolate inhibitory mAbs against the MMP-14 catalytic domain. One of the isolated mAbs (Fab 3A2) targets the S1' pocket of MMP-14 near the active site zinc and prevents proteolysis of a synthetic peptide substrate.

Targeting the active site, however, has other drawbacks. The active sites of all metzincins are structurally similar, as they all depend on the 3 histidines, the catalytic glutamic acid residue and a zinc ion. Consequently, mAbs that bind to the active site may suffer from poor selectivity. A solution to this is to target a region outside the active site cleft which is poorly conserved in close family members. For example, Botkjaer et al. (2016) isolated mAbs that bound to the MMP-14 Cat domain outside the active site cleft. Therefore, they did not inhibit cleavage of a small peptide substrate but still blocked pro-MMP-2 activation, collagen-film degradation and gelatin-film degradation, probably due to steric hindrance. The affinity-matured antibodies were able to inhibit collagen invasion by tumour-cells *in vitro.* They also reduced breast tumour growth and metastasis in a mouse xenograft model (MDA-MB-231).

In the MT-MMPs, a promising strategy is the targeting of the so called "MT-loop" in the Cat domain (^{163}PYAYIREG^{170} in MMP-14) (Fig 3), which lies distant from the active site but contains an exosite that is essential for TIMP-2 binding and, consequently, MMP-2 activation (Fernandez-Catalan et al., 1998; English et al., 2001). It also binds to integrin-rich cell adhesion complexes, an interaction necessary for locating MMP-14 to the leading edges of invading cells (Woskowicz et al. 2013). As expected, an antibody against the MT-loop (EP1264Y) did not prevent proteolysis of a short peptide substrate, gelatin or collagen but reduced cellular invasion (Woskowicz et al. 2013). The MT-loop is also recognised by another mAb, 9E8, which prevents TIMP-2 binding and MMP-2 activation (Ingvarsen et al., 2008). These mAbs show that targeting the MT-loop can be effective and because the MT-loop is also present in MMP-15, -16 and -24, but differs both in length and sequence, a similar approach can be applied to other MT-MMPs.

Besides the MT-loop, other loops are amenable to selective targeting by mAbs. For example, the V-B loop (residues 218-233 in MMP-14; Fig 3), is highly divergent among MMPs. A mAb was generated against the V-B loop in MMP-14 by immunization with a cyclic peptide containing the loop sequence (Udi et al., 2015). This mAb (LEM-2/15) exerts its inhibitory effect by inducing a conformational change in MMP-14, which results in a narrower substrate-binding cleft and, consequently, inhibits proteolysis of small synthetic peptides, gelatin and collagen type I. It has, however, only a moderate effect on MMP-2 activation (Galvez et al., 2001). It also inhibited endothelial cell invasion of collagen and fibrin gels and capillary tube formation in a Matrigel based assay, which highlights the important role of MMP-14 in angiogenesis (Galvez et al., 2001). Interestingly, LEM-2/15 was also tested in influenza/*S. pneumonia* coinfection models where it showed a synergistic effect with the antiviral drug Tamiflu both in a therapeutic and preventive mode (Talmi-Frank et al., 2016).
In addition to exosites in the Cat domain, metzincins often depend on exosites in ancillary domains to bind their substrates or co-factors. Several research labs have therefore targeted ancillary domains to generate function-specific inhibitory mAbs. The Hpx domain of MMP-14, for example, is important in unwinding the collagen triple helix for collagenolysis (Tam et al., 2002), as well as proMMP-2 activation (Itoh et al., 2001; Lehti et al., 2002) and CD44 shedding (Suenaga et al., 2005). The different functions of MMP-14 rely on distinct exosites, which can be targeted individually. For example, two scFv antibody fragments (named CHL and CHA), isolated against the Hpx domain of MMP-14, interfere specifically with the collagen-binding activity without affecting proMMP-2 activation (Basu et al., 2012). The interference with collagen binding translated into a strong attenuation of cellular invasion *in vitro* (Basu et al., 2012).
Interestingly, screening of inhibitory mAbs sometimes identifies antibodies that enhance, rather than inhibit the function of the target protease. Different mechanisms have been described that explain the “activation”. One example is a mAb against the Hpx domain of MMP-14 that enhances activation of MMP-2, without affecting collagenolytic activity (Ingvarsen et al., 2008). In this case, the divalent (IgG) antibody supported MMP-14 dimerization/ clustering on the cell surface. A derived Fab (monovalent) was therefore unable to replicate the increase in MMP-2 activation.

3. ADAMs

The ADAM (A Disintegrin and Metalloproteinase) family comprises 22 human proteins, 13 of which retain a functional Cat domain (Murphy, 2008). They are membrane-associated metzincins consisting of a prodomain, a Cat domain, a disintegrin-like (Dis) domain, a cysteine-rich (CysR) domain, an EGF (epidermal growth factor)-like domain (except ADAM-10 and ADAM-17), a transmembrane domain and a cytoplasmic tail (Figure 2). ADAMs are involved in ectodomain shedding of cell surface proteins and play a pivotal role in the extracellular regulation of cellular signalling.

3.1. ADAM-17

The best characterized ADAM family member is ADAM-17 , which is also known as TNF-α converting enzyme (TACE). It is involved in inflammation and cancer through its ability to cleave a variety of substrates such as TNF-α, EGF receptor (EGFR) ligands, and Notch1 (Murphy, 2008). The first mAb against ADAM-17, D1(A12), was developed using an ingenious two-step phage display strategy to circumvent cross reactivity with structurally

similar MMPs (Tape et al., 2011). The first step was to isolate a V_H chain that binds the Cat domain and then in the second step a V_L chain was selected for binding to the ancillary Dis/CysR region. The idea is that binding to the Cat domain blocks access of substrates to the active site whereas binding to the Dis/CysR region provides specificity. The resulting "cross-domain" human antibody is a potent inhibitor of ADAM-17 and does not inhibit its closest family member, ADAM-10 (Tape et al., 2011). Administration of D1(A12) in an animal model of ovarian cancer significantly inhibited tumour growth (Richards et al., 2012). It was also shown to reduce the *in vitro* proliferation of human breast tumours and squamous cell carcinoma of the head and neck (Huang et al., 2014; Caiazza et al., 2015). Unfortunately, D1(A12) is specific for human ADAM-17 which limited its preclinical applications to xenograft models. A second generation mAb, A9(B8), was therefore designed to cross react with mouse ADAM-17 (Kwok et al., 2014). This antibody suppressed the development of pancreatic cancer in mice overexpressing oncogenic Kras, by inhibiting both TNF-α and amphiregulin shedding (Ye et al., 2017). Another anti-ADAM17 antibody was developed by MedImmune, LLC. This mAb (MED13622) binds to the Cat domain only and acquires its specificity from binding to the highly variable loop between βIV and βV of the five-stranded β-sheet (Rios-Doria et al., 2015, Peng et al., 2016). It effectively inhibited cancer cell growth in the OE21 esophageal xenograft model, showing an additive effect with cetuximab (anti-EGFR). MED13622 was also used to ascertain that ADAM-17 can drive tumour proliferation through both EGFR-dependent and independent mechanisms. Moreover, proteomic studies that used this antibody to block shedding by ADAM-17 identified new ADAM-17 substrates (Rios-Doria et al., 2015).

3.2. Other ADAMs

ADAM-8 appears predominantly expressed on the surface of various immune cells, where it may act as a sheddase of cell-adhesion molecules (Gómez-Gaviro et al, 2007). Although the development of inhibitory mAbs against ADAM-8 has not been reported, one commercially available anti-ADAM-8 mAb (Mab1031) was tested for its effect on adhesion/migration of breast cancer cell lines and growth of xenograft breast tumours (Romagnoli et al., 2014). However, the ability of this mAb to inhibit proteolysis by ADAM-8 is not known.

ADAM-10 was targeted to study its proteolysis of Ephrins, which are membrane-bound ligands for tyrosine kinases of the erbB and Eph families. An anti-ADAM10 mAb (8C7) was generated against a distal substrate binding pocket in the CysR domain of ADAM-10 (Atapattu et al., 2012). It was shown to inhibit internalization of (cleaved) ephrin-A5 and Eph/ephrin-mediated cell function.

4. ADAMTS

The ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) family comprises 19 secreted metalloproteinases in humans (Dubail and Apte, 2015). ADAMTSs share a common domain composition consisting of a signal peptide, a prodomain, a metalloproteinase Cat domain, followed by the non-catalytic ancillary domains; a disintegrin-like (Dis) domain, a thrombospondin-type I motif (TS) domain, a CysR domain, a spacer (Sp) domain, and a variable number of TS domains in the C-terminus (with the exception of ADAMTS-4). Some of the ADAMTSs have further C-terminal domains (Figure 2).

4.1 ADAMTS-4 and -5

ADAMTS-1, -4, and -5 cleave proteoglycans such as aggrecan and versican, which play a structural role in many tissues. Aggrecan is a major component of articular cartilage where it provides the ability to resist compressive loads. This function is achieved by creating high osmolarity through hydration of the negatively-charged glycosaminoglycans attached to its protein core. Degradation of aggrecan is a clinical hallmark of degenerative joint disorders such as osteoarthritis (OA) and RA. Genetic knockout of ADAMTS-5 is sufficient to protect mice from cartilage destruction in OA or inflammatory arthritis models (Glasson et al., 2005; Stanton et al., 2005) and ADAMTS-5 is the most potent aggrecanase *in vitro* (Gendron et al., 2007). However, siRNA-mediated knock down of both ADAMTS-4 and ADAMTS-5 in human cartilage explants suggested that both enzymes are involved in aggrecan degradation (Song et al., 2007) and both have attracted considerable interest as a therapeutic target in arthritis.

Phage display has been used to obtain mAbs targeting exosites on ADAMTS-5 (Santamaria et al., 2015). Competition experiments with the endogenous inhibitor of aggrecanases, TIMP-3, and a small molecule zinc-chelating inhibitor (GM6001/Ilomastat) showed that all the isolated inhibitory mAbs recognized epitopes outside the active site cleft. One of these (2D3) targets the Cat/Dis domain and strongly inhibited aggrecanase activity both in unstimulated human chondrocyte cultures from healthy donors (Santamaria et al., 2015) and in OA cartilage explants (Yamamoto et al., 2017).

Interestingly, a mAb (1B7) that binds to the ADAMTS-5 Cat/Dis region did not inhibit its aggrecanolytic activity but blocked the endocytosis of ADAMTS-5 by LRP-1(low-density lipoprotein receptor-related protein-1). This caused human chondrocytes to accumulate ADAMTS-5, which increased the degradation of exogenously-added aggrecan (Santamaria et al., 2017).

The Sp domain of ADAMTS-5 is known to be important for aggrecan cleavage (Gendron et al., 2007), and this was confirmed by the inhibitory effect on aggrecan cleavage shown by a mAb (2B9) that specifically binds to the Sp domain (Santamaria et al., 2015). An anti-ADAMTS-5 mAb developed by Rottapharm (CRB0017), which was raised against a Sp-domain sequence, was also effective in a mouse model of OA when administered therapeutically (intra-articular route) (Chiusaroli et al., 2013).

Another mAb, 237-53, recognizes a common epitope in the TS-1 domain of both ADAMTS-4 and -5 and inhibited proteolysis of aggrecan in conditioned media of chondrocyte explants (Shiraishi et al., 2015). GlaxoSmithKline (GSK) has also raised several mAbs in mice against these two aggrecanases and tested their efficacy in preclinical models (Larkin et al., 2015). The two humanized anti-ADAMTS-5 mAbs, GSK2394002 and GSK2394000, recognized an epitope spanning the Cat and Dis domains, whereas of the two anti-ADAMTS-4 mAbs one bound to both the Cat and Dis domain and the other one to an epitope spanning the CysR and Sp domains. These mAbs have been extensively studied in the context of cartilage degradation using human OA explants (Larkin et al., 2015). The anti-ADAMTS-5 mAbs effectively inhibited aggrecan cleavage in unstimulated cultures, whereas the anti-ADAMTS-4 mAbs had a modest effect. However, both anti-ADAMTS-4 and -5 mAbs were effective in cytokine-stimulated cultures (Larkin et al., 2015). These data complement previous findings showing that ADAMTS-4 expression is induced under inflammatory conditions, whereas that of ADAMTS-5 is constitutive (Bau et al., 2002; Song et al., 2007; Shiraishi et al., 2015). Prophylactic, systemic treatment with anti-ADAMTS-5 mAbs imparted protection from cartilage degradation in a mouse OA model, thus phenocopying genetic deletion of *Adamts-5*

(Miller et al., 2016). One mAb (GSK2394002) also decreased aggrecan degradation in cynomolgus monkeys that were pre-screened for elevated circulating levels of aggrecan fragments (Larkin et al., 2015). However, this later study also highlighted potential challenges posed by ADAMTS-5 inhibition. An observation of subendocardial haemorrhage prompted monitoring of cardiovascular function, which showed a sustained increase in mean arterial pressure upon >3 mg/kg dosing and ST segment elevation in electrocardiograms upon dosing of >30 mg/kg (Larkin et al, 2014). These cardiovascular side effects of ADAMTS-5 inhibition may be mediated by modulation of the important physiological role of ADAMTS-5 as a versicanase in the cardiovascular system (Dupuis et al., 2011).

4.2 ADAMTS-13: lessons from autoantibodies.

ADAMTS-13 is a component of blood where it proteolyses ultra long (UL) Von Willebrand factor (VWF) multimers that are released into the circulation by endothelial cells. In patients with the autoimmune form of Thrombotic Thrombocytopenic Purpura (TTP), autoantibodies against ADAMTS-13 lower ADAMTS-13 levels and activity, which causes UL VWF multimers to remain in the circulation. UL VWF tend to spontaneously aggregate platelets, leading to thrombi that occlude small vessels.

Although ADAMTS-13 inhibition *in vivo* is detrimental, we include ADAMTS-13 in this review because extensive characterisations of antibodies against ADAMTS-13 have been instrumental in the understanding of ADAMTS-13 structure/function and its role in disease. Importantly, these findings may be instructive in the development of inhibitory antibodies against other metzincins, other ADAMTS family members in particular.

Extensive epitope mapping of patient autoantibodies has revealed that the CysR/Sp region of ADAMTS-13 is almost always targeted, whereas other domains are antigenic targets in some patients but not in others (Luken et al., 2005; Klaus et al., 2004; Zheng et al., 2010). Importantly, many patients had no antibodies against the Cat domain, highlighting the potency of antibodies against the ancillary domains. Despite these epitopes being very remote from the active site, the antibodies inhibit ADAMTS-13 activity directly by preventing ADAMTS-13 exosites in its ancillary domains to bind VWF (Thomas et al., 2015, de Groot et al., 2009; 2015; Pos et al., 2010). However, it is important to note that this interference with substrate binding is accompanied by autoantibody-mediated clearance of ADAMTS-13, which greatly reduces the ADAMTS-13 concentration in plasma of TTP patients (Thomas et al., 2015). The precise mechanism by which ADAMTS-13 antigen/antibody immune complexes are cleared is unknown but may involve Fc receptors on circulating macrophages or Kupffer cells.

A common epitope for autoantibodies in the Sp domain was shown to consist of two arginines, two tyrosines and a phenylalanine provided by neighbouring loop regions (Pos et al, 2011). Interestingly, these residues also appear to form an important exosite that binds VWF (Pos et al, 2010). Nature, therefore, seems to have provided us with a good illustration of inhibitory antibodies that target a distant exosite in an ancillary domain. The amino acids that make up the exosite/epitope are, as expected, poorly conserved in other ADAMTS family members, conferring specificity to both ADAMTS-13 and the autoantibodies. Consequently, for those ADAMTS family members that are drug targets, these loops in the Sp domain may be a good part of the molecule to target.

Paradoxically, one TTP patient has been described whose high-titre autoantibodies activated recombinant ADAMTS-13 3-fold when assayed with a VWF fragment (Muia et al., 2014). Although the development of TTP in this patient can be explained by clearance of ADAMTS-

13, the activating effect of the autoantibodies remained intriguing. Various mAbs have since been reported that enhance ADAMTS-13 activity. Out of the 31 anti-ADAMTS-13 mAbs that were screened, 11 showed a modest increase (≤2-fold) in VWF proteolysis, with some of them showing an additive effect when used in combination (≤4 fold) (Muia et al., 2014; Deforche et al., 2015). The activating mAbs bind to epitopes on the C-terminal domains of ADAMTS-13 (TSP5-CUB, Fig 2). *In vitro*, these C-terminal domains appear to shield an exosite, which is exposed when mAbs (or VWF C-terminal domains) bind and alter the ADAMTS-13 conformation (Muia et al., 2014).

5. Other metzincins

Pappalysin-1, also known as Pregnancy-associated plasma protein-A (PAPP-A) cleaves both insulin-like growth factor-binding protein 4 (IGFBP-4) and -5, thus regulating Insulin Growth Factor bioavailability. It consists of a laminin G-like module, Cat domain, five complement control protein (CCP) modules, and three Lin12-Notch repeats (LNR) of which two are inserted into the Cat domain and a third C-terminal to the CCP modules (Fig2). Interestingly, a scFv against the C-terminal LNR domain of PAPP-A inhibited proteolysis of IGFBP-4 much better than that of IGFBP-5 (Mikkelsen et al., 2008). This highlights the potential of mAbs against exosites in the ancillary domains to differentially inhibit cleavage of a particular substrate. A mAb with a similar epitope (mAb-PA) also inhibited tumour growth in lung and ovarian tumour xenografts (Mikkelsen et al., 2014; Becker et al., 2015).

Recently, mAbs against Decysin-1 (ADAMDEC1), an ADAM-like metzincin with unknown function, have been reported (Lund et al., 2017). ADAMDEC-1 consists only of a Cat domain followed by a short Dis domain (Mueller et al., 1997). Unusually, its catalytic zinc ion is coordinated by two histidines and an aspartic acid residue, rather than three histidine residues (Mueller et al., 1997). It was shown that mAbs targeting the Dis domain can have either an inhibitory or stimulating effect on caseinolytic activity (Lund et al., 2017), suggesting that even very short metzincins such as ADAMDEC1 are amenable to mAb-mediated "activation".

6. Conclusions and future directions

MAbs have an instrumental role in dissecting the functions of metzincins in biological systems and have great potential as therapeutic inhibitors in several diseases. As therapeutic inhibitors, mAbs have advantages and disadvantages compared to small molecule inhibitors. The major advantage of mAbs is their very high degree of selectivity, which has proven very difficult to achieve with small molecule inhibitors of metzincins. Other advantages are that metabolic drug-drug interactions are rare and toxicity is generally low. Compared to small molecule inhibitors, most mAbs will also cover a larger surface contact area, which makes them more suited to disrupt high affinity interactions between metzincins and their substrates/cofactors through direct competition or steric hindrance.

A major drawback of therapeutic mAbs in general is that they need to be injected, either subcutaneously or intravenously. Moreover, their usage is restricted to extracellular targets. Although metzincins operate mostly extracellularly, it was recently discovered that several MMPs are active intracellularly (Ali et al., 2010; Jobin et al., 2017). The inability of mAbs to reach the cytoplasm could potentially explain a lack of efficacy of mAbs in some *in vivo* studies. Specific targeting of intracellular activities of MMPs could potentially be achieved

using transfection with antibody constructs that are modified for intracellular localisation ("intrabodies") (Stocks, 2005; Marschall et al. 2015).
An important consideration for future mAb development is that various metzincins use exosites (in Cat and ancillary domains) to bind co-factors and substrates, which can therefore be targeted to inhibit the enzyme. Several examples of this have been given in this review (e.g. MMP-14, ADAM-17, and ADAMTS-5). Autoantibodies against ADAMTS-13 also highlight the inhibitory potential of antibodies against ancillary domains through both disruption of substrate binding and clearance of immune complexes. For those future metzincin targets that have multiple physiological substrates, substrate-specific inhibition may be achieved by mabs against exosites that mediate susbtrate specificity. Examples of such mAbs have already been described (e.g. Mikkelsen et al., 2008). Here lies also an important task for metzincin research, i.e. to identify the exosite(s) that dictate substrate specificity.
So far, the development of mAbs has been limited to few metzincin targets (Table 1) and Gilead Science's anti-MMP-9 mAb, GS-5745 appears currently the only anti-metzincin mAb tested in clinical trials. As research progresses and more becomes known about the roles that metzincins play in various diseases, this will change. There are already several metzincins with known roles in disease, which may be future targets (Overall and Kleifeld, 2006; Yang et al., 2017). A prominent example is ADAMTS-7, which is emerging as a potential target in cardiovascular disease (Arroyo and Andres, 2015).
In summary, there is a lot of potential for the development of highly specific mAb inhibitors against MMPs, ADAMTSs and ADAMSs. The increasing use of therapeutic mAbs in general suggests that the expansion of this field will ultimately lead to anti-metzincin mAbs able to modify detrimental roles in disease.

Nomenclature of Targets and Ligands
Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

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Conflict of interest
The authors declare that they have no conflict of interest.

References

Ager EI, Kozin SV, Kirkpatrick ND, Seano G, Kodack DP, Askoxylakis V et al. (2015) Blockade of MMP14 activity in murine breast carcinomas: implications for macrophages, vessels, and radiotherapy. J Natl Cancer Inst. 107(4).

Alexander SPH, Doriano Fabbro, Eamonn Kelly, Neil V Marrion, John A Peters, Elena Faccenda, et al (2017), THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Enzymes Vol: 174, Pages: S272–S359. DOI: 10.1111/bph.13877

Ali MA, Cho WJ, Hudson B, Kassiri Z, Granzier H, Schulz R (2010) Titin is a target of matrix metalloproteinase-2: implications in myocardial ischemia/reperfusion injury. Circulation. 122(20):2039-47.

Appleby TC, Greenstein AE, Hung M, Liclican A, Velasquez M, Villaseñor AG, et al. (2017) Biochemical characterization and structure determination of a potent, selective antibody inhibitor of human MMP9. J Biol Chem. 292(16):6810-6820.

Arroyo AG, Andrés V (2015), ADAMTS7 in cardiovascular disease: from bedside to bench and back again? Circulation. 131(13):1156-9.

Atapattu L, Saha N, Llerena C, Vail ME, Scott AM, Nikolov DB, et al. (2012) Antibodies binding the ADAM10 substrate recognition domain inhibit Eph function. J Cell Sci 125(Pt 24):6084-93.

Basu B, Correa de Sampaio P, Mohammed H, Fogarasi M, Corrie P, Watkins NA, et al. (2012) Inhibition of MT1-MMP activity using functional antibody fragments selected against its hemopexin domain. Int J Biochem Cell Biol. 44(2):393-403.

Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T (2002) Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro. Arthritis Rheum 46 (10):2648-57.

Becker MA, Haluska P Jr, Bale LK, Oxvig C, Conover CA (2015) A novel neutralizing antibody targeting pregnancy-associated plasma protein-a inhibits ovarian cancer growth and ascites accumulation in patient mouse tumorgrafts. Mol Cancer Ther. 14(4):973-81.

Bode W, Gomis-Rüth FX, Stöcker W (1993) Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the ‘metzincins’. FEBS Lett. 331 (1-2):134-40.

Botkjaer KA, Kwok HF, Terp MG, Karatt-Vellatt A, Santamaria S, McCafferty J, et al. (2016) Development of a specific affinity-matured exosite inhibitor to MT1-MMP that efficiently inhibits tumor cell invasion in vitro and metastasis in vivo. Oncotarget 7(13):16773-92.

Bu Z, Pan Y, Shang B, Cao Z, Zhou Q, Ruan C (2012) SZ-117, a monoclonal antibody against matrix metalloproteinase-2 inhibits tumor cell-mediated angiogenesis. Hybridoma 31(1):63-7.

Caiazza F, McGowan PM, Mullooly M, Murray A, Synnott N, O'Donovan N, et al. (2015) Targeting ADAM-17 with an inhibitory monoclonal antibody has antitumour effects in triple-negative breast cancer cells. Br J Cancer 112:1895-903.

Cauwe B, Opdenakker G (2010) Intracellular substrate cleavage: a novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases. Crit Rev Biochem Mol Biol. 45(5):351-423.

Chiusaroli R, Visentini M, Galimberti C, Casseler C, Mennuni L, Covaceuszach S, et al. (2013) Targeting of ADAMTS-5's ancillary domain with the recombinant mAb CRB0017 ameliorates disease progression in a spontaneous murine model of osteoarthritis. Osteoarthritis Cartilage 21, 1807-1810

Coussens LM, Fingleton B, Matrisian LM (2002), Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295 (5564):2387-92.

Deforche L, Roose E, Vandenbulcke A, Vandeputte N, Feys HB, Springer TA, et al. (2015) Linker regions and flexibility around the metalloprotease domain account for conformational activation of ADAMTS-13. J Thromb Haemost. 13(11):2063-75.

de Bruyn M, Vandooren J, Ugarte-Berzal E, Arijs I, Vermeire S, Opdenakker G (2016) The molecular biology of matrix metalloproteinases and tissue inhibitors of metalloproteinases in inflammatory bowel diseases. Crit Rev Biochem Mol Biol. 51(5):295-358.

de Groot R, Bardhan A, Ramroop N, Lane DA, Crawley JT (2009) Essential role of the disintegrin-like domain in ADAMTS13 function. Blood. 113(22):5609-5616.

de Groot R, Lane DA, Crawley JT (2015) The role of the ADAMTS13 cysteine-rich domain in VWF binding and proteolysis. Blood. 125(12):1968-1975.

Demeestere D, Dejonckheere E, Steeland S, Hulpiau P, Haustraete J, Devoogdt N, et al. (2016) Development and Validation of a Small Single-domain Antibody That Effectively Inhibits Matrix Metalloproteinase 8. Mol Ther. 24(5):890-902.

Devy L, Huang L, Naa L, Yanamandra N, Pieters H, Frans N, et al. (2009), Selective inhibition of matrix metalloproteinase-14 blocks tumor growth, invasion, and angiogenesis. Cancer Res 69 (4):1517-26.

Dubail J, Apte SS (2015) Insights on ADAMTS proteases and ADAMTS-like proteins from mammalian genetics. Matrix Biol. 44-46:24-37.

Dupuis LE, McCulloch DR, McGarity JD, Bahan A, Wessels A, Weber D, et al. (2011), Altered versican cleavage in ADAMTS5 deficient mice; A novel etiology of myxomatous valve disease. Dev Biol. 357 (1):152-64.

Emara M, Wozniak M (2010) In vitro inhibition of human MMP-2 collagenolytic and gelatinolytic activity by neutralizing monoclonal antibodies. Immunol Invest. 39(8):833-48.

English WR, Holtz B, Vogt G, Knauper V, Murphy G (2001) Characterization of the role of the 'MT-loop': an eight-amino acid insertion specific to progelatinase A (MMP2) activating membrane-type matrix metalloproteinases. J Biol Chem 276: 42018–42026.

Fanjul-Fernandez M, Folgueras AR, Cabrera S, Lopez-Otin C (2010) Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models. Biochimica et biophysica acta. 1803 (1):3–19.

Fernandez-Catalan C, Bode W, Huber R, Turk D, Calvete JJ, Lichte A, et al. (1998) Crystal structure of the complex formed by the membrane type 1-matrix metalloproteinase with the tissue inhibitor of metalloproteinases-2, the soluble progelatinase A receptor. EMBO J. 17(17):5238-48.

Gaggar A, Li Y, Weathington N, Winkler M, Kong M, Jackson P, et al. (2007), Matrix metalloprotease-9 dysregulation in lower airway secretions of cystic fibrosis patients. Am J Physiol Lung Cell Mol Physiol. 293(1):L96-L104.

Galvez BG, Matıas-Roman S, Albar JP, Sanchez-Madrid F, Arroyo AG (2001) Membrane type 1-matrix metalloproteinase is activated during migration of human endothelial cells and modulates endothelial motility and matrix remodeling. J. Biol. Chem. 276, 37491–37500.

Gendron, C, Kashiwagi M, Lim NH, Enghild JJ, Thogersen IB, Hughes C, et al. (2007) Proteolytic activities of Human ADAMTS-5. Comparative studies with ADAMTS-4. J Biol Chem. 282 (25):18294-306.

Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, et al. (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 434 (7033):644-8.

Gómez-Gaviro M, Domínguez-Luis M, Canchado J, Calafat J, Janssen H, Lara-Pezzi E, et al. (2007), Expression and regulation of the metalloproteinase ADAM-8 during human neutrophil pathophysiological activation and its catalytic activity on L-selectin shedding. J Immunol. 178(12):8053-63.

Gomis-Rüth FX, Botelho TO, Bode W (2012) A standard orientation for metallopeptidases. Biochim Biophys Acta. 1824(1):157-63.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 46: D1091-D1106.

Höyhtya M, Hujanen E, Turpeenniemi-Hujanen T, Thorgeirsson U, Liotta LA, Tryggvason K (1990) Modulation of type-IV collagenase activity and invasive behavior of metastatic human melanoma (A2058) cells in vitro by monoclonal antibodies to type-IV collagenase. Int J Cancer. 46(2):282-6.

Hu J, Van den Steen PE, Sang QX, Opdenakker G (2007) Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov.6(6):480–98.

Huang Y, Benaich N, Tape C, Kwok HF, Murphy G (2014) Targeting the sheddase activity of ADAM17 by an anti-ADAM17 antibody D1(A12) inhibits head and neck squamous cell carcinoma cell proliferation and motility via blockage of bradykinin induced HERs transactivation. Int J Biol Sci 10:702-14.

Ingvarsen S, Madsen DH, Hillig T, Lund LR, Holmbeck K, Behrendt N, et al. (2008) Dimerization of endogenous MT1-MMP is a regulatory step in the activation of the 72-kDa gelatinase MMP-2 on fibroblasts and fibrosarcoma cells. Biol Chem 389:943–53.

Itoh Y, Takamura A, Ito N, Maru Y, Sato H, Suenaga N, et al. (2001) Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. EMBO J 20:4782–93.

Itoh Y (2015), Membrane-type matrix metalloproteinases: Their functions and regulations. Matrix Biol. 44-46:207-23.

Jobin PG, Butler GS, Overall CM (2017) New intracellular activities of matrix metalloproteinases shine in the moonlight. Biochim Biophys Acta. 1864(11 Pt A):2043-2055.

Kaneko K, Williams RO, Dransfield DT, Nixon AE, Sandison A, Itoh Y (2016) Selective Inhibition of Membrane Type 1 Matrix Metalloproteinase Abrogates Progression of Experimental Inflammatory Arthritis: Synergy With Tumor Necrosis Factor Blockade. Arthritis Rheumatol. 68(2):521-31.

Klaus C, Plaimauer B, Studt JD, Dorner F, Lämmle B, Mannucci PM, et al. (2004), Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. Blood. 103(12):4514-9.

Kwok HF, Botkjaer KA, Tape CJ, Huang Y, McCafferty J, Murphy G (2014) Development of a 'mouse and human cross-reactive' affinity-matured exosite inhibitory human antibody specific to TACE (ADAM17) for cancer immunotherapy. Protein Eng Des Sel. 27(6):179-90

Larkin J, Lohr T, Elefante L, Shearin J, Matico R, Su JL, et al. (2014) The highs and lows of translational drug development: antibody-mediated inhibition of ADAMTS-5 for osteoarthritis disease modification. Osteoarthritis and Cartilage 22, S883.

Larkin J, Lohr TA, Elefante L, Shearin J, Matico R, Su JL, et al. (2015) Translational development of an ADAMTS-5 antibody for osteoarthritis disease modification. Osteoarthritis Cartilage. 23(8):1254-66.

Lauwereys M, Arbabi Ghahroudi M, Desmyter A, Kinne J, Hölzer W, De Genst E, et al. (1998), Potent enzyme inhibitors derived from dromedary heavy-chain antibodies. EMBO J. 17(13):3512-20.

Lehti K, Lohi J, Juntunen MM, Pei D, Keski-Oja J (2002) Oligomerization through hemopexin and cytoplasmic domains regulates the activity and turnover of membrane-type 1 matrix metalloproteinase. J Biol Chem 277:8440–8.

Lopez T, Nam DH, Kaihara E, Mustafa Z, Ge X (2017) Identification of highly selective MMP-14 inhibitory Fabs by deep sequencing. Biotechnol Bioeng. 2017 Jun;114(6):1140-1150.

Luken BM, Turenhout EA, Hulstein JJ, Van Mourik JA, Fijnheer R, Voorberg J (2005) The spacer domain of ADAMTS13 contains a major binding site for antibodies in patients with thrombotic thrombocytopenic purpura. Thromb Haemost. 93(2):267-274.

Lund J, Elimar Bitsch AM, Grønbech Rasch M, Enoksson M, Troeberg L, Nagase H, et al. (2017) Monoclonal antibodies targeting the disintegrin-like domain of ADAMDEC1 modulates the proteolytic activity and enables quantification of ADAMDEC1 protein in human plasma. MAbs. 2017 Nov 29:1-11.

Marchant DJ, Bellac CL, Moraes TJ, Wadsworth SJ, Dufour A, Butler GS, et al. (2014). A new transcriptional role for matrix metalloproteinase-12 in antiviral immunity. Nat Med 20: 493–502.

Marschall AL, Dübel S, Böldicke T (2015), Specific in vivo knockdown of protein function by intrabodies. MAbs. 7(6):1010-35.

Marshall DC, Lyman SK, McCauley S, Kovalenko M, Spangler R, Liu C, et al. (2015) Selective allosteric inhibition of MMP9 is efficacious in preclinical models of ulcerative colitis and colorectal cancer. PLoS One 10, e0127063

Martens E, Leyssen A, Van Aelst I, Fiten P, Piccard H, Hu J, et al. (2007) A monoclonal antibody inhibits gelatinase B/MMP-9 by selective binding to part of the catalytic domain and not to the fibronectin or zinc binding domains. Biochim Biophys Acta. 1770(2):178-86.

Mikkelsen JH, Gyrup C, Kristensen P, Overgaard MT, Poulsen CB, Laursen LS, et al. (2008) Inhibition of the proteolytic activity of pregnancy-associated plasma protein-A by targeting substrate exosite binding. J Biol Chem. 283(24):16772-80.

Mikkelsen JH, Resch ZT, Kalra B, Savjani G, Kumar A, Conover CA, et al. (2014) Indirect targeting of IGF receptor signaling in vivo by substrate-selective inhibition of PAPP-A proteolytic activity. Oncotarget. 5(4):1014-25

Miller MC, Manning HB, Jain A, Troeberg L, Dudhia J, Essex D, et al. (2009) Membrane type 1 matrix metalloproteinase is a crucial promoter of synovial invasion in human rheumatoid arthritis Arthritis Rheum. 60:686–697.

Miller RE, Tran PB, Ishihara S, Larkin J, Malfait AM (2016) Therapeutic effects of an anti-ADAMTS-5 antibody on joint damage and mechanical allodynia in a murine model of osteoarthritis. Osteoarthritis Cartilage. 24(2):299-306.

Mueller CGF, Rissoan M, Salinas B, Ait-Yahia S, Ravel O, Bridon J, et al. Polymerase Chain Reaction Selects a Novel Disintegrin Proteinase from CD40-Activated Germinal Center Dendritic Cells. J Exp Med. 1997;187(5):655–63.

Muia J, Zhu J, Gupta G, Haberichter SL, Friedman KD, Feys HB, et al. (2014), Allosteric activation of ADAMTS13 by von Willebrand factor. Proc Natl Acad Sci U S A 111(52):18584-9.

Murphy G. (2008), The ADAMs: signalling scissors in the tumour microenvironment. Nat Rev Cancer. 8(12):929-41.

Murphy G, Nagase H (2008) Progress in matrix metalloproteinase research. Mol Aspects Med 29, 290–308.

Naito S, Takahashi T, Onoda J, Yamauchi A, Kawai T, Kishino J, et al. (2012) Development of a neutralizing antibody specific for the active form of matrix metalloproteinase-13. Biochemistry. 51(44):8877-84.

Nam DH, Rodriguez C, Remacle AG, Strongin AY, Ge X (2016) Active-site MMP-selective antibody inhibitors discovered from convex paratope synthetic libraries. Proc Natl Acad Sci U S A. 113(52):14970-14975.

Nam DH, Fang K, Rodriguez C, Lopez T, Ge X (2017) Generation of inhibitory monoclonal antibodies targeting matrix metalloproteinase-14 by motif grafting and CDR optimization. Protein Eng Des Sel. 30(2):113-118.

Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y (1997) Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 272:2446–51.

Overall CM, Kleifeld O (2006) Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer. 6(3):227-39.

Paemen L, Martens E, Masure S, Opdenakker G (1995), Monoclonal antibodies specific for natural human neutrophil gelatinase B used for affinity purification, quantitation by two-site ELISA and inhibition of enzymatic activity, Eur. J. Biochem. 234, 759–765.

Peng L, Cook K, Xu L, Cheng L, Damschroder M, Gao C, et al. (2016) Molecular basis for the mechanism of action of an anti-TACE antibody. MAbs. 8(8):1598-1605

Pos W, Crawley JT, Fijnheer R, Voorberg J, Lane DA, Luken BM (2010) An autoantibody epitope comprising residues R660, Y661, and Y665 in the ADAMTS13 spacer domain identifies a binding site for the A2 domain of VWF. Blood. 115(8):1640-1649.

Pos W, Sorvillo N, Fijnheer R, Feys HB, Kaijen PH, Vidarsson G, et al. (2011), Residues Arg568 and Phe592 contribute to an antigenic surface for anti-ADAMTS13 antibodies in the spacer domain. Haematologica 96(11):1670-7.

Pruijt JF, Fibbe WE, Laterveer L, Pieters RA, Lindley IJ, Paemen L, et al. (1999) Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). Proc Natl Acad Sci U S A. 96(19):10863-8.

Ramos-DeSimone N, Moll UM, Quigley JP, French DL (1993) Inhibition of matrix metalloproteinase 9 activation by a specific monoclonal antibody. Hybridoma. 12(4):349-63.

Remacle AG, Cieplak P, Nam DH, Shiryaev SA, Ge X, Strongin AY (2017) Selective function-blocking monoclonal human antibody highlights the important role of membrane type-1 matrix metalloproteinase (MT1-MMP) in metastasis. Oncotarget. 8(2):2781-2799.

Richards FM, Tape CJ, Jodrell DI, Murphy G (2012) Anti-tumour effects of a specific anti-ADAM17 antibody in an ovarian cancer model in vivo. PLoS One 7:e40597.

Rios-Doria J, Sabol D, Chesebrough J, Stewart D, Xu L, Tammali R, et al. (2015) A monoclonal antibody to ADAM17 inhibits tumor growth by inhibiting EGFR and non-EGFR-mediated pathways. Mol Cancer Ther 14:1637-49.

Romagnoli M, Mineva ND, Polmear M, Conrad C, Srinivasan S, Loussouarn D, et al. (2014) ADAM8 expression in invasive breast cancer promotes tumor dissemination and metastasis. EMBO Mol Med 6(2):278-94.

Sandborn WJ, Bhandari BR, Fogel R, Onken J, Yen E, Zhao X, et al. (2016), Randomised clinical trial: a phase 1, dose-ranging study of the anti-matrix metalloproteinase-9 monoclonal

antibody GS-5745 versus placebo for ulcerative colitis. Aliment Pharmacol Ther. 44(2):157-69.

Santamaria S, Yamamoto K, Botkjaer K, Tape C, Dyson MR, McCafferty J, et al. (2015) Antibody-based exosite inhibitors of ADAMTS-5 (aggrecanase-2). Biochem J. 471(3):391-401.

Santamaria S, Fedorov O, McCafferty J, Murphy G, Dudhia J, Nagase H, et al. (2017) Development of a monoclonal anti-ADAMTS-5 antibody that specifically blocks the interaction with LRP1. MAbs. 9(4):595-602.

Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, et al. (1994) A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 370:61–5.

Sela-Passwell N, Kikkeri R, Dym O, Rozenberg H, Margalit R, Arad-Yellin R, et al. (2011), Antibodies targeting the catalytic zinccomplex of activated matrix metalloproteinases show therapeutic potential. Nat Med. 18 (1):143-7.

Shimanovich I, Mihai S, Oostingh GJ, Ilenchuk TT, Bröcker EB, Opdenakker G, et al. (2004), Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. J Pathol. 204(5):519-27.

Shiraishi A, Mochizuki S, Miyakoshi A, Kojoh K, Okada Y. (2015), Development of human neutralizing antibody to ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2). Biochem Biophys Res Commun. 469(1):62-69.

Shiryaev SA, Remacle AG, Golubkov VS, Ingvarsen S, Porse A, Behrendt N, et al. (2013) A monoclonal antibody interferes with TIMP-2 binding and incapacitates the MMP-2-activating function of multifunctional, pro-tumorigenic MMP-14/MT1-MMP. Oncogenesis. 2:e80.

Song RH, Tortorella MD, Malfait AM, Alston JT, Yang Z, Arner EC, et al. (2007) Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. Arthritis Rheum. 56 (2):575-85.

Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, et al. (2005), ADAMTS-5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 434 (7033):648-52.

Stocks M (2005) Intrabodies as drug discovery tools and therapeutics. Curr Opin Chem Biol. 9(4):359-65.

Suenaga N, Mori H, Itoh Y, Seiki M (2005) CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase. Oncogene 24:859–68.

Tallant C, García-Castellanos R, Baumann U, Gomis-Rüth FX (2010), On the relevance of the Met-turn methionine in metzincins. J Biol Chem. 285(18):13951-7

Talmi-Frank D, Altboum Z, Solomonov I, Udi Y, Jaitin DA, Klepfish M, et al. (2016) Extracellular Matrix Proteolysis by MT1-MMP Contributes to Influenza-Related Tissue Damage and Mortality. Cell Host Microbe. 20(4):458-470.

Tam EM, Wu YI, Butler GS, Stack MS, Overall CM (2002) Collagen binding properties of the membrane type-1 matrix metalloproteinase (MT1-MMP) hemopexin C domain. The ectodomain of the 44-kDa autocatalytic product of MT1-MMP inhibits cell invasion by disrupting native type I collagen cleavage. J Biol Chem 277:39005–14.

Tape CJ, Willems SH, Dombernowsky SL, Stanley PL, Fogarasi M, Ouwehand W, et al. (2011), Cross-domain inhibition of TACE ectodomain. Proc Natl Acad Sci U S A. 108 (14):5578-83.

Thomas MR, de Groot R, Scully MA, Crawley JT (2015) Pathogenicity of Anti-ADAMTS13 Autoantibodies in Acquired Thrombotic Thrombocytopenic Purpura. EBioMedicine. 2(8):942-952.

Udi Y, Grossman M, Solomonov I, Dym O, Rozenberg H, Moreno V, et al. (2015) Inhibition mechanism of membrane metalloprotease by an exosite-swiveling conformational antibody. Structure. 23(1):104-15.

Woskowicz AM, Weaver SA, ShitomiY, Ito N, Itoh,Y. (2013) MT-LOOP-dependent localization of membrane type I matrix metalloproteinase (MT1-MMP) to the cell adhesion complexes promotes cancer cell invasion. J. Biol. Chem. 288, 126–137.

Yamamoto K, Trad A, Baumgart A, Hüske L, Lorenzen I, Chalaris A, et al. (2012) A novel bispecific single-chain antibody for ADAM17 and CD3 induces T-cell-mediated lysis of prostate cancer cells. Biochem J 445:135-44.

Yamamoto K, Santamaria S, Botkjaer KA, Dudhia J, Troeberg L, Itoh Y, et al. (2017) Inhibition of Shedding of Low-Density Lipoprotein Receptor-Related Protein 1 Reverses Cartilage Matrix Degradation in Osteoarthritis. Arthritis Rheumatol. 69(6):1246-1256.

Yang CY, Chanalaris A, Troeberg L (2017), ADAMTS and ADAM metalloproteinases in osteoarthritis - looking beyond the 'usual suspects'. Osteoarthritis Cartilage. 25(7):1000-1009.

Ye J, Yuen SM, Murphy G, Xie R, Kwok HF (2017) Anti-tumor effects of a 'human & mouse cross-reactive' anti-ADAM17 antibody in a pancreatic cancer model in vivo. Eur J Pharm Sci. Eur J Pharm Sci. 110:62-69.

Zheng XL, Wu HM, Shang D, Falls E, Skipwith CG, Cataland SR, et al. (2010), Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura.
Haematologica. 95(9):1555-62

Figures

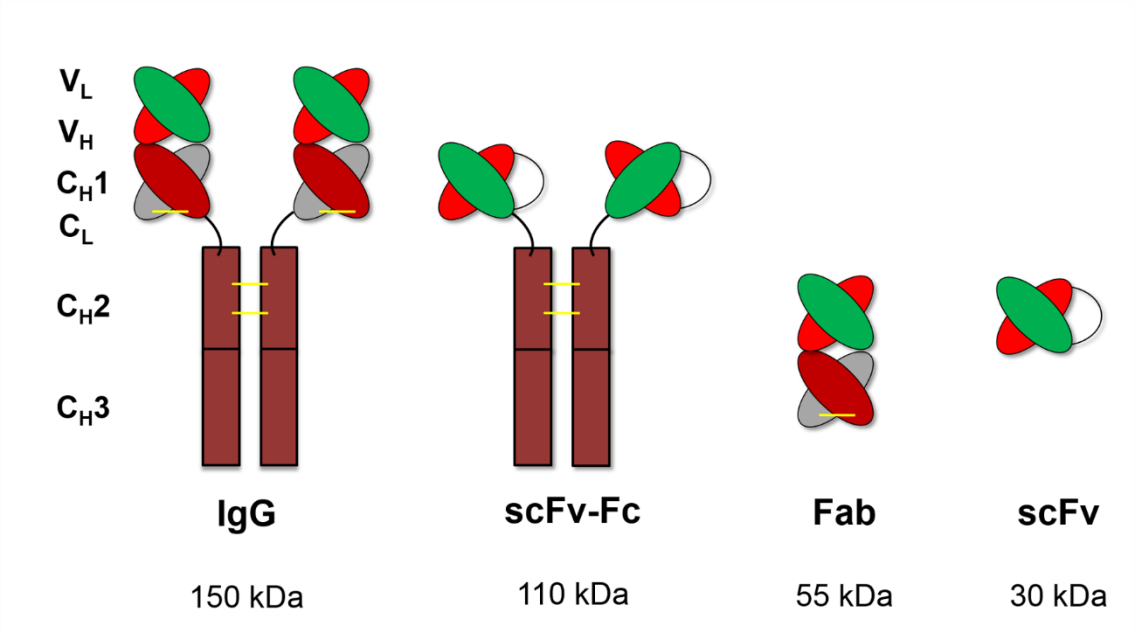


Figure 1: Schematic illustration of IgG and IgG fragments. IgGs are bivalent and consist of two heavy and two light chains, made of variable (V_H and V_L) and constant (C_H and C_L) regions. The heavy chain constant region is divided into C_H1, C_H2 and C_H3. The antigen binding site is composed of six hypervariable complementarity determining regions (CDRs), three in the variable region of the heavy (V_H) and light (V_L) chains. The "fragment crystallisable" (Fc) region is composed of the C_H2 and C_H3 constant regions of the antibody and mediates interactions with cell surface (Fc) receptors and the complement system. The antigen-binding fragment (Fab) is composed of one constant and one variable region of each of the heavy and light chains (monovalent). It can be produced from an intact IgG by digestion with papain or it can be expressed recombinantly. The scFv consists of single V_H and V_L regions connected by a flexible linker and is therefore monovalent. The scFv-Fc is bivalent and consists of two scFvs connected to the Fc region of IgG. Yellow lines indicate disulfide bonds.

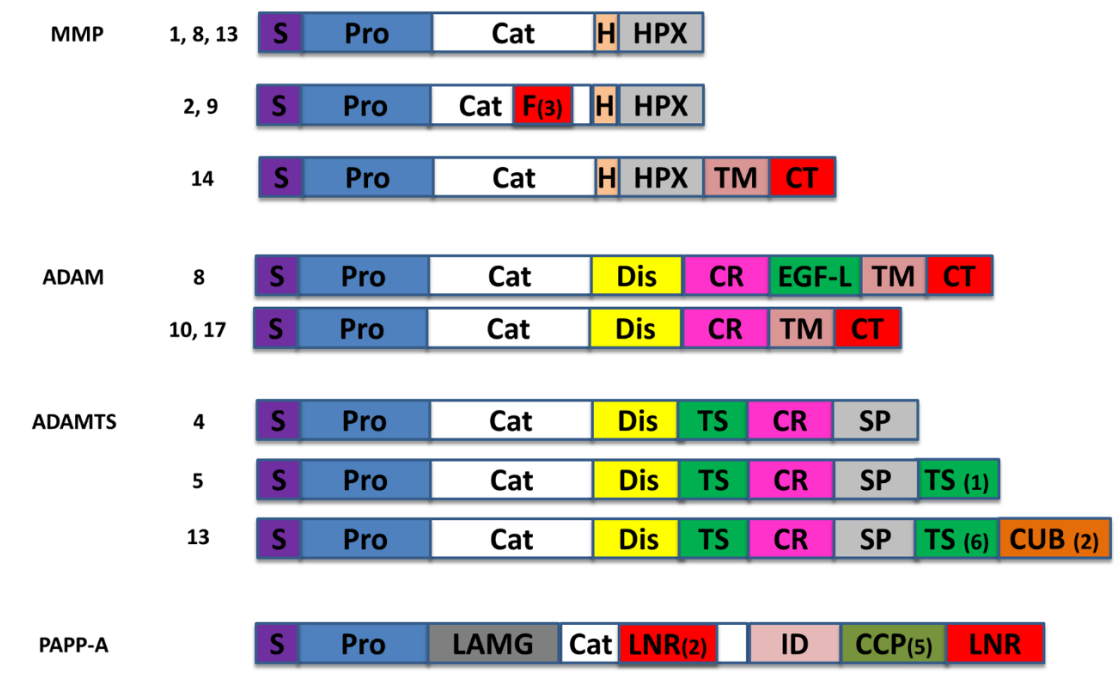


Figure 2: Domain organisation of metzincins discussed in this review. S: signal peptide, Pro: prodomain, Cat: catalytic domain, F: fibronectin type II repeats, H: hinge region, HPX: hemopexin domain, TM: transmembrane domain, CT: c-terminal tail, Dis: disintegrin-like domain, CR: cysteine-rich domain, EGF-L: EGF-like domain, TS: Thrombospondin type-1 like repeat, SP: spacer domain, CUB: complement c1r/c1s, uegf, bmp1 domain, LAMG: laminin G-like domain, LNR: lin12-notch repeats, ID: interdomain region, CCP: complement control protein. For tandem repeats, the number of repeats is shown in brackets. Note that the disintegrin-like domains of ADAMs and ADAMTSs are structurally very different.

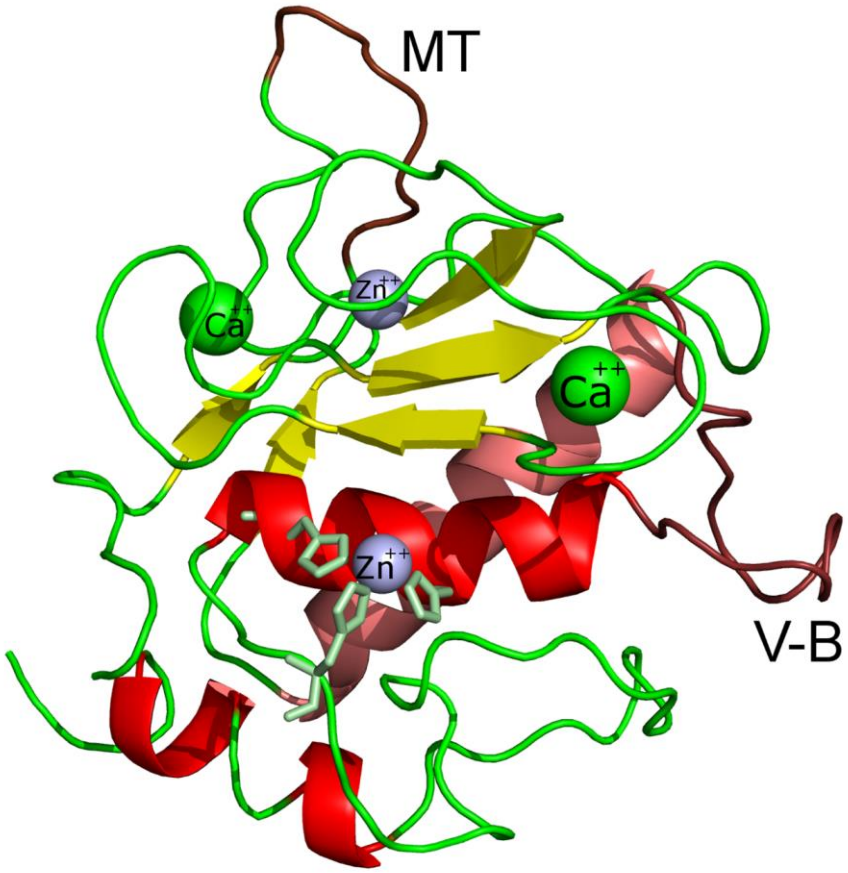


Figure 3: Structure of the catalytic domain of MMP-14. The catalytic domains of members of the metzincin clan have a characteristic fold consisting of an active site groove that runs approximately along the axis of the central alpha-helix beneath it, here shown horizontally at the centre of the domain. This active site helix also provides two of the three histidines that bind the catalytic zinc ion. Structural stability of MMP-14 is provided by a second zinc ion (purple circle) and two calcium ions (green circles). Indicated are the membrane-type (MT) loop (residues 163-170) and the VB-loop (residues 218-233), which have been used for selective targeting by mAbs. PDB ID: 1BQQ (Fernandez-Catalan et al, 1998).

Table 1: Properties of mAbs developed against metzincin targets

mAb	Format	Target	Epitope	K_D (nM)	K_i (nM)	Effect	Effective Concentration In vitro/ex vivo (nM)	Effective concentration in vivo (mg/kg)	Reference
		MMPs							
F2-1-11 G8-25-5	IgG	MMP-2	ND	ND	ND	Inhibits catalytic activity	ND	ND	Emara & Wozniak, 2010
SZ-117	IgG	MMP-2	Fibronectin type 2	ND	ND	Inhibits catalytic activity	1000	ND	Bu et al. 2012
Nb14	Nb	MMP-8	Cat	0.24-1.7	4400[1,2] 19500[3]	Inhibits catalytic activity	ND	ND	Demeestere et al. 2016
SDS3	IgG	MMP-9	Catalytic zinc	200	1000	Inhibits catalytic activity	ND	1-5	Sela-Passwell et al. 2011
SDS4	IgG	MMP-9	Catalytic zinc	20	54	Inhibits catalytic activity	ND	ND	Sela-Passwell et al. 2011
5F3, 6B2, 6D2, 6D7	IgG	MMP-9	ND	ND	ND	Inhibit catalytic activity	667	ND	Höyhtya et al., 1990
4B7	IgG	MMP-9	ND	ND	ND	Increases catalytic activity	667	ND	Höyhtya et al., 1990
REGA-3G12	IgG	MMP-9	Cat	2.1	ND[2,3]	Inhibits catalytic activity	ND	ND	Paemen et al., 1995 Martens et al., 2007 Pruijt et al., 1999 Shimanovich et al., 2004
	scFv						16670 (0.5mg/mL)	1-2	
6-6B	IgG	MMP-9	ND	ND	Complete inhibition at 1ug/ml	Inhibits enzyme activation	ND	ND	Ramos-DeSimone et al.,1993
GS-5745	IgG	MMP-9	Cat	0.17	0.22[1]	Inhibits catalytic activity and enzyme activation	ND	15	Marshall et al., 2015* Appleby et al., 2017
				0.008-0.04 (pro) 3.3 (Cat)	0.3-1.3[1,2]				
								0.3-5	Sandborn et al., 2016
14D10	IgG	MMP-13	Active site	0.33	12[1] 20[3]	Inhibits catalytic activity	260	ND	Naito et al., 2012
DX-2400	IgG	MMP-14	Cat	ND	0.8	Inhibits catalytic activity	6.7-67	10-30	Devy et al.,2009 Ager et al., 2015
							ND	0.2-100	Kaneko et al., 2016
							10-500	20-40	
3A2	Fab	MMP-14	Cat	4.8	9.7[1]	Inhibits catalytic activity	200-250	ND	Nam et al., 2016
1F8	Fab	MMP-14	Cat	8.3	110	Inhibits catalytic activity	ND	ND	Nam et al., 2017
R2C5	Fab	MMP-14	Cat	15	50[1]	Inhibits catalytic activity	ND	ND	Lopez et al., 2017
R2C7	Fab	MMP-14	Cat	27	100[1]	Inhibits catalytic activity	ND	ND	Lopez et al., 2017

R2C14	Fab	MMP-14	Cat	6.1	10[1]	Inhibits catalytic activity	ND	ND	Lopez et al., 2017
E2_C6 A4_7	scFc-Fv	MMP-14	Cat	0.11-4	100[1,2]	Inhibits catalytic activity and pro-MMP-2 activation	3-500	5	Botkjaer et al., 2016
3A2	Fab	MMP-14	Cat	8	18[1]	Inhibits catalytic activity and pro-MMP-2 activation	200-500	10-15	Remacle et al., 2017
$LOOP_{ab}$	IgG	MMP-14	MT-loop	ND	ND	Inhibits cell localization	100	ND	Woskowicz et al.
9E8	IgG	MMP-14	MT-loop	ND	0.6	Inhibits proMMP-2 activation	10-650	ND	Ingvarsen et al. 2013 Shiryaev et al.
LEM-2/15	Fab	MMP-14	V-B loop	2.3	45[1]	Inhibits catalytic activity	5000	ND 3	Galvez et al., 2001 Udi et al. Talmi-Frank et al., 2016
mAb-1	Fab IgG	MMP-14	Hemopexin	ND	ND	Induces MMP-14 dimerization	330	ND	Ingvarsen et al. 2008
CHL	scFv	MMP-14	Hemopexin	169	ND	Inhibits proMMP-2 activation	1560-5000	ND	Basu et al. 2012
CHA	scFv	MMP-14/15	Hemopexin	10.7	ND	Inhibits proMMP-2 activation and CD44 shedding	420-5000	ND	Basu et al. 2012
		ADAMs							
Mab1031	IgG	ADAM-8	Cat/Dis	ND	ND	Inhibits catalytic activity	133	0.5-1.5	Romagnoli et al., 2014
8C7	IgG	ADAM-10	CysR	93	ND	Inhibits catalytic activity Inhibits ephrin endocytosis	667-2670	ND	Atapattu et al., 2012
D1(A12)	Fab IgG IgG	ADAM17	Cat/Dis/Cys	0.46	0.89[1] 74[5]	Inhibits catalytic activity	8.6[1] 8-11[1] 200	 10	Tape et al., 2011 Tape et al., 2011 Richards et al., 2012
A9(B8)	IgG	ADAM17	Cat	0.33	1.19	Inhibits catalytic activity	200-250[1] 200-250	- 10	Kwok et al., 2014 Ye et al., 2017
A300E-BiTE	Bispecific scFV	ADAM-17/CD-3	CysR	ND	ND	Induces T-cell mediated lysis of cancer cells	6.67	ND	Yamamoto et al., 2012
MEDI3622	IgG	ADAM-17	Cat	0.039	3.1[1]	Inhibits catalytic activity	0.9[1] 400	1-30	Rios-Doria et al., 2015 Peng et al., 2016
		ADAMTSs							
7E8.1E3	IgG	ADAMTS-4	Cat/Dis	0.25	0.035[1,4]	Inhibits catalytic activity	670	ND	Larkin et al., 2015
7C7.1H1	IgG	ADAMTS-4	CysRich/Sp	0.29	0.048[1,4]	Inhibits catalytic activity	670	ND	Larkin et al., 2015
GSK2394000	IgG	ADAMTS-5	Cat/Dis	0.21	11[1,4]	Inhibits catalytic activity	670	ND	Larkin et al., 2015
GSK2394002	IgG	ADAMTS-5	Cat/Dis	0.038	0.083[1,4]	Inhibits catalytic activity	670	10-16 30-300 (Cynomolgus	Larkin et al., 2015

								m.)	
								10	Miller et al., 2016
2B9	scFc-Fv	ADAMTS-5	Sp	6.6	90-140 [1,4]	Inhibits activity against macromolecular substrates	ND	ND	Santamaria et al., 2015
2D3	scFc-Fv	ADAMTS-5	Cat/Dis	3.9	2.5[1] 8-90[1,4]	Inhibits catalytic activity	10-100	ND	Santamaria et al., 2015
									Yamamoto et al., 2017
1B7	scFc-Fv	ADAMTS-5	Cat/Dis	70	NI	Inhibits endocytosis	250 100	ND	Santamaria et al., 2017
CRB0017	IgG	ADAMTS-5	Sp	2.2	ND	Inhibits aggrecanase activity	20	12μg/knee	Visintin et al., 2012 Chiusaroli et al., 2013
237-53	Fab	ADAMTS-4	TS-1	12	80[1,4]	Inhibits aggrecanase activity	5.4	ND	Shiraishi et al., 2015
237-53	Fab	ADAMTS-5	TS-1	1.5	ND	Inhibits aggrecanase activity	5.4	ND	Shiraishi et al., 2015
			OTHER METZINCINS						
PAC1	scFv	PAPP-A	LNR3	0.25	1.2	Inhibit cleavage of IGFBP-4 but not IGFBP-5	ND	ND	Mikkelsen et al., 2008
PAC2 1/41	IgG	PAPP-A	LNR3	0.097	0.14	Inhibits catalytic activity	1-0.001	10-30	Mikkelsen et al., 2014
								30	Becker et al., 2015
129	Fab	ADAMDEC1	Dis	9.3	ND ND ND	Inhibit catalytic activity Increase catalytic activity[6]	ND	ND	Lund et al., 2017
177	Fab		Dis	1.4			ND	ND	
111	Fab		Dis	0.38			ND	ND	

[1]IC_{50} value; [2], measured against gelatin; [3], measured against collagen;[4], measured against aggrecan; 5, measured against TNF-α; [6], measured against casein. NI, not inhibitory, ND, not determined, only single concentrations tested. *, GS-5745 is the humanized version of AB0041

Nb, nanobody, single variable domain derived from heavy-chain-only antibodies of *Camelidae*