

Expert Opinion

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Tackling EGFR signaling with TACE antagonists: a rational target for metalloprotease inhibitors in cancer

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TNF- α converting enzyme (TACE/ADAM17) is a transmembrane metalloprotease that plays a key role in the cleavage and mobilization of receptor ligands that are initially synthesized as membrane-tethered precursors. For many years, attention has focused on the role of TACE-dependent TNF- α cleavage in arthritis and, more recently, it has become apparent that TACE also plays an important role in regulating epidermal growth factor receptor activity in several tumor types. This review presents the background to these findings and a rationale for the continued development of TACE inhibitors for the treatment of epidermal growth factor receptor-dependent epithelial tumors.

Keywords: ADAM17, cancer, EGFR, metalloprotease inhibitor, sheddase

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1. Introduction

The epidermal growth factor receptor (EGFR) is the prototypic receptor tyrosine kinase and its role in normal development, homeostasis and tumor biology has been explored extensively over the past quarter century since its discovery in 1984 [1]. There are four members of this receptor family (EGFR, ErbB2, ErbB3 and ErbB4) and these are bound by a large family of ligands that includes amphiregulin, betacellulin, EGF, epigen, epiregulin, Heparin-binding EGF-like growth factor (HB-EGF) and Transforming growth factor- α (TGF- α) [2]. Ligand binding results in the formation of homo- or heterodimers between the receptors and, depending on the precise dimer formed, transphosphorylation occurs and signaling complexes are recruited to the cytoplasmic domain. Activation of the members of this receptor family typically leads to the activation of a series of signaling cascades that are frequently hyperactivated in cancer, including the Raf-MEK-ERK pathway, the PI3-kinase pathway and the phospholipase C pathway (Figure 1). Depending on the nature of the dimer, the cellular context and on the amplitude and duration of the signaling elicited, a variety of outcomes may occur, but generally activation of these receptors leads to enhanced proliferation and/or cell survival.

The EGFR signaling pathway is activated in a substantial proportion of epithelial tumors, although there are some tissue-specific biases in how this pathway activation is achieved. The receptors themselves can be amplified and overexpressed in many different tumor types, including non-small-cell lung cancer [3], glioblastomata [4], breast [5], pancreatic [6] and ovarian cancers [7], and squamous cell carcinoma of the head and neck [8]. Alternatively, positively acting downstream components of the pathway may sustain activating mutations.

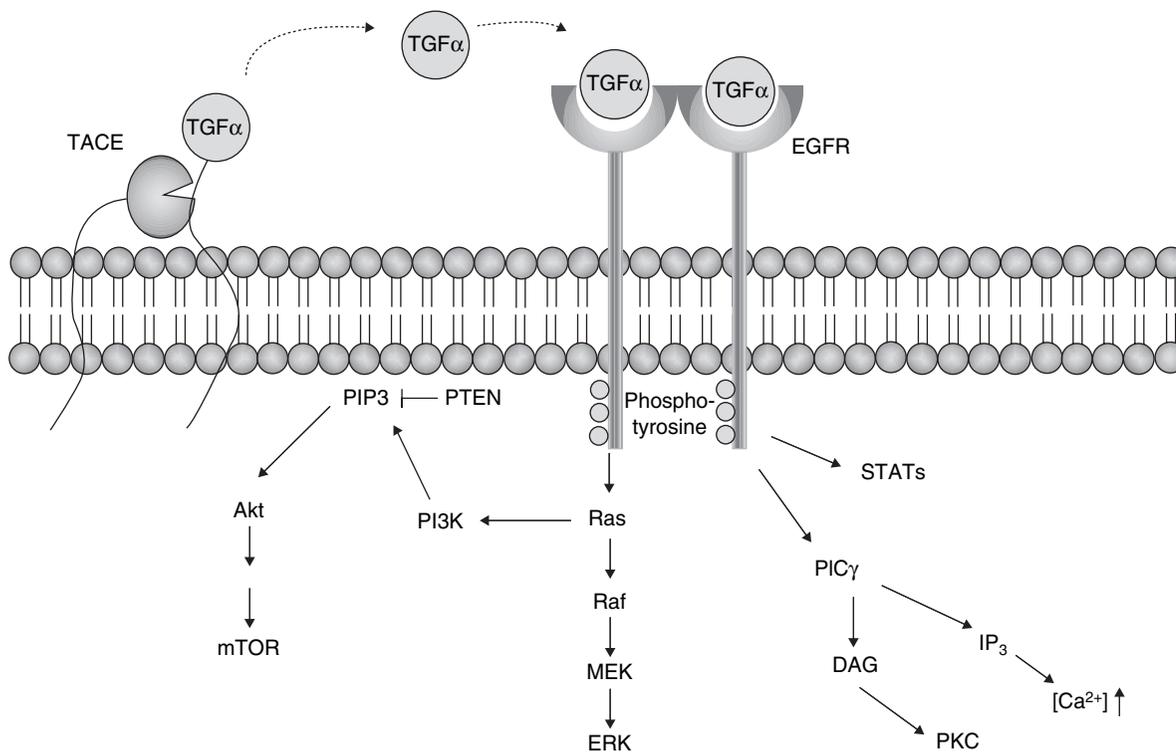


Figure 1. A schematic representation of the EGFR signaling pathway. In response to ligand binding, receptors dimerize and phosphorylate each other. These phospho-tyrosine residues then recruit a series of adapter proteins that activate signaling cascades that promote cell proliferation, protein synthesis and survival. The transmembrane protease TACE plays a key role in initiating this signal by mobilizing EGFR ligands from the cell surface.

DAG: Diacylglycerol; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; IP3: Inositol-1,4,5-triphosphate; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositide 3-kinase; PKC: Protein kinase C; PLC: Phospholipase C; STAT: Signal transducer and activator of transcription; TACE: TNF- α converting enzyme; TGF: Transforming growth factor.

Activating mutations in genes encoding Ras proteins are very frequent in pancreatic, thyroid and colorectal cancer [9], and mutations that activate the PI3-kinase gene are found in as many as a third of all breast cancers [10-12]. Similarly, proteins that serve to attenuate the amplitude of signaling downstream of the receptors can be mutationally inactivated, transcriptionally silenced or deleted. Phosphatase and tensin homolog deleted on chromosome ten (PTEN), which antagonizes the PI3-kinase pathway is a frequent target for loss-of-function mutation [13]. The MAPK phosphatase, DUSP6, which acts as a feedback inhibitor on the Ras-Raf-MAPK pathway, is also frequently silenced by promoter methylation in pancreatic cancer [14,15].

Another important and very common mechanism of pathway hyperactivation is by overexpression of one or more growth factor receptor ligands, either by the tumor cells themselves or by non-malignant cells of the tumor micro-environment, such as macrophages. Ligand overexpression is a common feature of many tumors [16-19]. Ligands for the EGFR are typically produced as transmembrane precursors, many of which require proteolytic shedding in order to activate their cognate receptor and stimulate proliferation.

Members of the ADAM family of metal-dependent proteases, most prominently ADAM10 and ADAM17 (TACE), have been implicated in the shedding of these ligands, suggesting that targeting the proteolytic activity of these enzymes may prove to be an effective means of blocking inappropriate EGFR pathway activation in tumor cells.

2. Existing approaches to target EGFR

Deregulation of the EGFR signaling pathway, at some level, is a common feature of tumors from all epithelial tissues. Existing therapeutic approaches use small molecules or blocking antibodies to specifically target members of the ErbB receptor family [20]. For a list of agents presently approved or under investigation to target these receptors, see Table 1. In addition, small molecules have been developed to target various intracellular components of the signaling cascade, for example sorafenib (BAY-43-9006), which targets Raf, SF-1126, which targets PI3-kinase [21] and temsirolimus (CCI-779), which targets the mammalian target of rapamycin (mTOR) [22]. Fortuitously, some of these kinase

Table 1. Examples of ErbB receptor antagonists.

	Generic name	Target	Company
Small molecules	Gefitinib	EGFR	Astra Zeneca
	Erlotinib	ErbB2	Genentech
	Lapatinib	EGFR/ErbB2	GlaxoSmithKline
Antibodies	Cetuximab	EGFR	ImClone
	Trastuzumab	ErbB2	Genentech
	Panitumumab	EGFR	Amgen
	Pertuzumab	ErbB2*	Genentech

*Pertuzumab binds ErbB2 and prevents its dimerization with other ErbB family members.

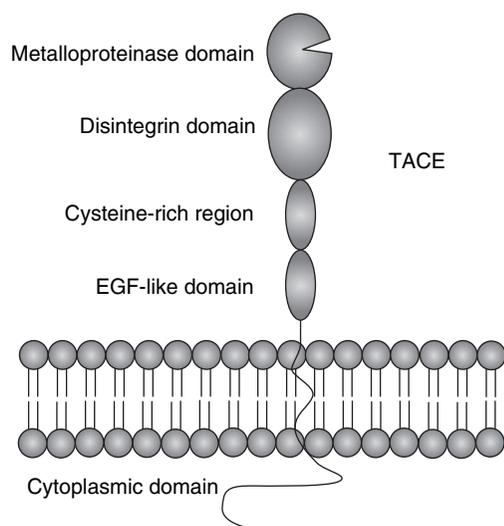


Figure 2. A schematic representation of the domain structure of active TACE at the cell surface after proteolytic removal of the inhibitory pro-domain. The zinc-binding metalloprotease domain contains the active site of the enzyme.

EGF: Epidermal growth factor; TACE: TNF- α converting enzyme.

inhibitors have useful overlapping specificities against other cancer-relevant kinases. For example, in addition to B-Raf and C-Raf, sorafenib inhibits vascular endothelial growth factor receptors (VEGFRs) 1 and 2 and platelet derived growth factor receptor β (PDGFR β) and so, by blocking angiogenesis, may exert a number of additional anti-tumor effects [23]. Of the small-molecule EGFR inhibitors, both gefitinib and erlotinib were FDA approved for the treatment of non-small cell lung cancer [24-27] although these have only proven effective in small populations of patients; particularly those carrying tumors bearing mutations in EGFR [28,29]. Erlotinib has received approval for metastatic pancreatic cancer in combination with gemcitabine, although again, the actual survival benefit was

miniscule; the median survival time of patients given gemcitabine and erlotinib versus gemcitabine alone was only 10 days longer [30]. Based on a series of trials, a small molecule dual inhibitor of EGFR and ErbB2, lapatinib has been approved in combination with capecitabine for patients with ErbB2-overexpressing advanced or metastatic breast cancer [31,32]. The blocking antibody, cetuximab has been FDA approved for the treatment of metastatic colorectal cancer and in squamous cell carcinoma of the head and neck [33]. Panitumumab has been approved for chemotherapy-refractory colorectal cancer based on a trial that showed that the median extension in progression-free survival in treated versus untreated patients was just 5 days [34]. Overall, given the prevalence of EGFR pathway aberrations in cancer, the relatively small numbers of responses to these billion dollar drugs have been disappointing so far. Even for the indications for which these drugs have been approved, the response at the population level has been extremely modest [35].

3. TACE – a key metalloprotease in cancer and arthritis

TACE/ADAM17 was identified as the cell surface protease responsible for the shedding of TNF- α , an important pro-inflammatory cytokine in rheumatoid arthritis [36,37]. As a chronic disease afflicting many millions of people, rheumatoid arthritis is an attractive indication for pharmaceutical companies to develop agents that will alleviate the pain and suffering caused by this disease. Such drugs might be expected to be taken continually by sufferers for a number of decades. Thus, the identification of the enzyme responsible for the shedding of TNF- α from the cell surface was met with great interest and a number of companies began developing small-molecule inhibitors of TACE for the treatment of rheumatoid arthritis.

TACE is a member of the ADAM family of metalloproteases [38]. Members of this family of proteases share a common domain organization, having an N-terminal signal peptide, followed by a pro-domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an EGF-like domain and transmembrane and cytosolic domains (Figure 2). The pro-domain inhibits the proteolytic activity of TACE until it is cleaved off by a pro-protein convertase, such as furin.

Transgenic mouse models were generated to better understand the physiologic roles of TACE. Surprisingly, mice in which the zinc binding domain of TACE was deleted exhibited several phenotypes in common with mice in which the EGFR or EGFR ligands were knocked out. These mice had curly vibrissae, a failure in eyelid fusion, defects in the morphogenesis of several organs and died perinatally [39]. Mice null for TGF- α are born with open eyes and a distinctive derangement of the hair follicles which leads to a wavy coat and whiskers [40,41]. Mice null for TACE also have

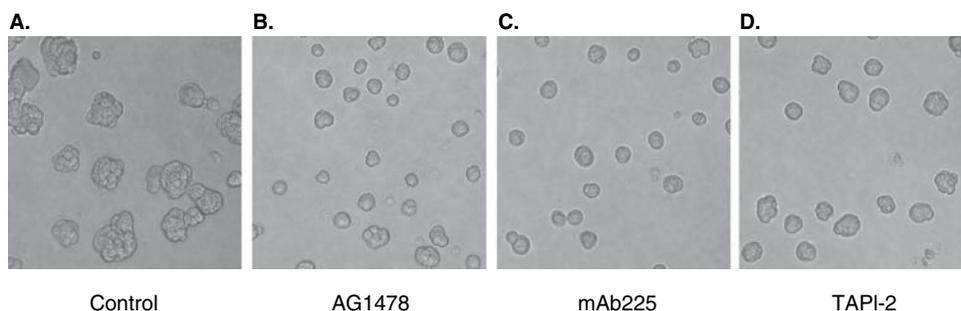


Figure 3. Three-dimensional Matrigel culture models of human T4-2 breast cancer cells. Untreated cells form aggressive proliferative colonies, whereas cells treated with small molecule inhibitors of EGFR (AG-1478, 80 nM) or EGFR blocking antibodies (mAb225, 4 μ g/ml) respond by forming organized growth-arrested colonies. Inhibition of TACE in this model (TAPI-2, 20 μ M) also reverts the malignant phenotype of these cells.

EGFR: Epidermal growth factor receptor; TACE: TNF- α converting enzyme.

heart defects similar to those found in HB-EGF knockout mice [42]. These data suggested that, in addition to TNF- α cleavage, TACE was likely to play a key role in the regulation of EGFR signaling. A comprehensive analysis of mouse embryonic fibroblasts null for various combinations of ADAM genes provided clear evidence of roles for TACE in the shedding of amphiregulin, TGF- α , epiregulin and HB-EGF [43]. In the same study, ADAM10 was shown to be the key sheddase for EGF and betacellulin. TACE was also reported to be a key regulator of amphiregulin shedding during mammary gland morphogenesis [44].

A role for TACE in the shedding of EGFR ligands in cancer cells has now been reported by many laboratories, including ours. TACE-dependent shedding of amphiregulin was implicated in EGFR pathway activation and cell proliferation in head and neck squamous cell carcinoma [45,46]. Both Src and G-protein-coupled receptor activity were implicated as being upstream of TACE activation in these studies. Other workers have identified a role for MAPK phosphorylation of the cytoplasmic tail of TACE in the regulation of its activity [47]. TACE inhibition in a model of renal cell carcinoma led to reduced shedding of TGF- α , with concomitant downregulation of EGFR phosphorylation, cell migration and proliferation [48]. Studies in a breast cancer model from the author's laboratory showed that TACE inhibition potently suppressed shedding of the EGFR ligands, amphiregulin and TGF- α , downregulated EGFR pathway activity and phenocopied EGFR inhibition by reverting the malignant phenotype of these breast cancer cell lines in a three-dimensional culture model [49]. In this model, malignant breast epithelial cells proliferate in disorganized colonies on a layer of Matrigel (Figure 3A) and, if treated with a drug to which they are sensitive (e.g., EGFR inhibitors, Figures 3B and C), their morphology can be reverted to that of a non-malignant breast acinus [50-52]. Here it can clearly be seen that TACE inhibition (Figure 3D) effectively phenocopies EGFR inhibition.

Importantly, this study demonstrated that TACE inhibition effectively suppressed EGFR ligand shedding in several breast cancer cell lines and the examination of a large panel of breast tumors [53] revealed that TACE and TGF- α are frequently co-expressed in the so-called 'basal' or 'triple-negative' subset of human breast cancers [49]. These data are summarized in Table 2. This group of breast tumors is clinically important as it has a very poor prognosis and, being typically negative for estrogen receptor, progesterone receptor and ErbB2, it is unaffected by the existing targeted therapies for breast cancer [54-57]. The survival curves of groups of patients in this study with tumors of each class is shown in Figure 4. The basal class of tumors also frequently expresses EGFR [58,59], thus suggesting the likelihood that a TACE-dependent TGF- α to EGFR autocrine loop may be a common feature of this disease. These data suggest the attractive hypothesis that inhibition of EGFR, TACE or both in this patient population may prove to be a useful treatment option.

4. Metalloprotease inhibitors in the cancer clinic: a litany of disappointment

Although the inhibitors used in many of these studies were not exclusively specific to TACE, the key results have been replicated using specific siRNAs and knockout mice. Based on these data, TACE can now be considered a validated therapeutic target with a well-defined mechanistic role in promoting tumor cell proliferation by modulating the availability of ligands for the EGFR and related receptors. The pathways downstream of these receptors (Figure 1) play key roles in regulating cell proliferation and survival in many epithelial tumors. As such, one might expect that over the past few years pharmaceutical companies would have been making strenuous efforts to bring compounds to clinical trials to target EGFR signaling in this way. As described above, there are many competing drugs that inhibit this pathway

Table 2. Analysis of the distribution of the EGFR ligands, amphiregulin and TGF- α , and TACE in 295 human breast cancer cases.

	Basal		ERRB2		Luminal A		Luminal B		Normal		n						
AREG	UQ	1/46	2.2%	13/49	26.5%	23/88	26.1%	20/81	24.7%	17/31	54.8%	74					
	IQR	17/46	37.0%	p < 0.0001	24/49	49.0%	NS	54/88	61.4%	p < 0.05	42/81	51.9%	NS	10/31	32.3%	p < 0.001	147
	LQ	28/46	60.9%		12/49	24.5%	11/88	12.5%	19/81	23.5%		4/31	12.9%		74		
TGF- α	UQ	31/46	67.4%		13/49	26.5%	13/88	14.8%	10/81	12.3%	7/31	22.6%		74			
	IQR	13/46	28.3%	p < 0.0001	28/49	57.1%	NS	39/88	44.3%	p < 0.05	46/81	56.8%	p < 0.05	21/31	67.7%	NS	147
	LQ	2/46	4.3%		8/49	16.3%	36/88	40.9%	25/81	30.9%		3/31	9.7%		74		
TACE	UQ	26/46	56.5%		14/49	28.6%	15/88	17.0%	14/81	17.3%	5/31	16.1%		74			
	IQR	17/46	37.0%	p < 0.0001	26/49	53.1%	NS	42/88	47.7%	p < 0.05	47/81	58.0%	NS	15/31	48.4%	NS	147
	LQ	3/46	6.5%		9/49	18.4%	31/88	35.2%	20/81	24.7%		11/31	35.5%		74		

A summary of the distribution of the expression levels of two EGFR ligands, amphiregulin and TGF- α , and TACE in 295 human breast cancer cases. Cases are stratified into groups by their gene expression profiles [56] and by whether they express high, medium or low levels of the genes of interest. Of the tumors that express high levels of TACE and TGF- α , significantly more belong to the basal subclass. Reproduced with modification from [49].

IQR: Interquartile range; LQ: Lower quartile; NS: Not significant; TACE: TNF- α converting enzyme; UQ: Upper quartile.

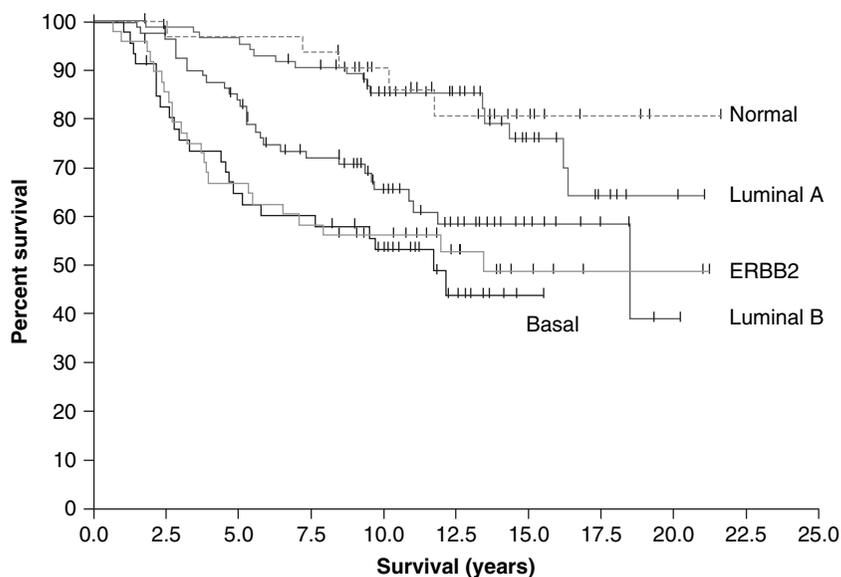


Figure 4. Kaplan–Meier survival analysis of 295 patients [53] stratified into distinct groups by the gene expression profiles of their tumors. Patients carrying basal tumors (which often have high levels of TGF- α and TACE, see **Table 2**) have a poor outcome with existing therapies.

at the level of the receptor or downstream signaling intermediates. There have been no previous methods identified of targeting this pathway above the level of the receptor and TACE inhibitors represent an attractive means by which to do so. Nevertheless, most researchers have proven reticent to embrace this new challenge, perhaps because many have acute and painful memories of the ‘boom and bust’ of metalloprotease inhibitors in the 1990s.

The matrix metalloprotease (MMP) inhibitor ‘boom’ began with the demonstration that metalloproteases were important for cancer cell invasion and metastasis [60]. This elicited intense interest among academic and industrial researchers in the 1980s and early 1990s. Large numbers of studies (reviewed in [61]) reported that MMPs were highly expressed in many tumor types and correlated with poor prognosis. Several pharmaceutical companies began to develop compounds to inhibit the (very few) metalloproteases known at that time. The fundamental expectation underlying this work was that inhibition of MMPs would prevent the invasion and metastasis of tumor cells *in vivo* and provide a clinical benefit to cancer patients. A succession of *in vitro* and preclinical studies supported this hypothesis. These agents proved efficacious in xenograft models of ovarian [62], breast [63,64], colorectal [65], gastric [66], lung [67] and prostate [68] cancers. In addition, exciting data from more realistic transgenic (as opposed to xenograft) mouse models of pancreatic cancer demonstrated that batimastat (BB-94) effectively inhibited tumor growth [69].

These exciting preclinical data propelled a succession of MMP inhibitors into human clinical trials. Batimastat, which was used extensively in preclinical studies, was rapidly

superseded by marimastat, due to the latter’s superior oral availability [70]. Other agents tested in advanced clinical trials included prinomastat (AG-3340) [67], tanomastat (BAY-12-9566) [71] and BMS-275291 [72]. These compounds were tested in Phase III trials in patients with pancreatic, gastric, glioblastoma, small-cell and non-small-cell lung, prostate and ovarian tumors (summarized in [73,74]) and the trials were uniformly unsuccessful. Trials of one agent, tanomastat, were suspended prematurely when treated patients showed significantly poorer survival rates than patients receiving placebo. Another issue, not evident in the preclinical rodent models, was a pronounced musculo-skeletal syndrome that limited the amount of these drugs that could be delivered to patients.

5. TACE inhibitors

Because of the initial strong interest in TACE inhibition as a means of treating arthritis, several companies began to develop TACE inhibitors for this purpose. Based on the recently emerging data of the key role that TACE plays in EGFR pathway activation, it is reasonable to propose that some of these tested compounds may have utility in EGFR activity suppression in cancer. Several compounds were identified and have been tested in animal models and trials in human patients have also been performed.

5.1 Preclinical studies

Although all of the compounds are potent inhibitors of TACE, the extent to which they inhibit other metalloproteases varies significantly. These data are summarized in **Table 3**.

Table 3. IC₅₀ values (nM) for various TACE-targeting agents against a panel of metalloproteases.

Protease	Marimastat	TMI-1	TMI-2	Ro-32-7315	GW-3333	INCB-3619
TACE	12	8.4	2	5.2	40	14
MMP-1	4	6.6	2471	500	19	> 5000
MMP-2	3	4.7	ND	250	14	35
MMP-3	30	ND	ND	190	20	3500
MMP-7	20	26	961	250	ND	> 5000
MMP-8	ND	ND	35	25	4	ND
MMP-9	< 5	12	777	100	16	304
MMP-12	< 5	ND	ND	11	ND	17
MMP-13	ND	3	96	110	7	ND
MMP-14	< 5	26	582	ND	ND	772
ADAM8	ND	ND	ND	ND	ND	1000
ADAM9	247	ND	ND	ND	ND	> 5000
ADAM10	78	ND	1090	ND	ND	22
ADAM33	155	ND	ND	ND	ND	1036

Summarized from [75-78,80].

ADAM: A disintegrin and metalloproteinase; ND: Not determined; MMP: Matrix metalloproteinase; TACE: TNF- α converting enzyme.

Ro-32-7315 (Roche) is a potent orally active TACE inhibitor with an *in vitro* IC₅₀ of 5.2 nM [75]. It caused a dose-dependent suppression of lipopolysaccharide (LPS)-induced TACE-dependent TNF- α cleavage in cultured cells and in the circulation of LPS-treated rats.

Wyeth and Amgen have developed a number of compounds, including TMI-1, TMI-2 and TMI-5. TMI-1 is a potent TACE inhibitor (IC₅₀ = 8.4 nM), but inhibits several other MMPs with IC₅₀ values \leq 26 nM. It inhibits LPS-dependent TNF- α cleavage in cell based assays and in LPS-treated mice, and showed efficacy in a series of mouse models of rheumatoid arthritis [76]. TMI-2 is also a potent TACE inhibitor (IC₅₀ = 2 nM), but is considerably more selective than TMI-1 [77]. In tests against a large panel of MMPs, only MMPs 8 and 13 were significantly inhibited (with IC₅₀ values of 35 and 96 nM, respectively). TMI-2 also prevented TACE-dependent TNF- α cleavage in cultured cells and in LPS-treated mice and antiarthritic efficacy was demonstrated in both mouse and rat models.

GW-3333, developed by GlaxoSmithKline, is also a reasonably effective TACE inhibitor (IC₅₀ = 40 nM), but unfortunately has considerable potency against several other MMPs. Like the other compounds tested, GW-3333 inhibits the function of TACE *in vitro* and *in vivo* and showed efficacy in a rat adjuvant arthritis model [78].

INCB-3619 and INCB-7839 have been developed by Incyte Corporation [79], the only pharmaceutical company that is presently focused on the use of ADAM inhibitors to affect ErbB signaling pathways. INCB-3619 potently inhibits ADAM10 and TACE (IC₅₀ values 22 and 14 nM, respectively) and only inhibited MMPs 2, 12 and 14 from

a large panel of MMPs [80]. INCB-3619 blocked the ADAM-dependent shedding of amphiregulin, heregulin, TGF- α , HB-EGF and EGF [80] in addition to the extracellular domain of HER2 [81]. In mouse models, INCB-3619 reduced levels of circulating TGF- α and inhibited the growth of a series of xenografted cancer cell lines [80-82]. Importantly, INCB-3619 frequently had a synergistic effect with other targeted therapies in these studies.

A series of specific TACE inhibitors (IC₅₀ < 10 nM) that do not inhibit MMPs 1, 2, 9 and 13 have been reported by Bristol-Myers Squibb [83], but these remain to be evaluated in animal models.

5.2 Human trials

The majority of human clinical trials of TACE inhibitors performed so far have been in the context of arthritis research. From a cancer perspective, however, it is encouraging from these studies that TACE inhibitors are well tolerated in human subjects and can effectively inhibit TACE proteolytic activity at clinically achievable doses. This favorable toxicity profile contrasts sharply with earlier metalloproteinase inhibitors, and although TACE activity seems to be required for successful embryonic mouse development [39], it seems that this enzyme can be inhibited in adult humans without significant adverse effects.

Ro-32-7315 was tested in healthy volunteers. Blood samples were removed from drug- and placebo-treated individuals and treated with LPS. TNF- α cleavage was suppressed in the blood samples from those individuals who received the drug [75]. TMI-1 was tested on synovial explants from rheumatoid arthritis patients and successfully reduced

TNF- α production in this *ex vivo* setting [76,77]. TMI-5 was evaluated in clinical trials of rheumatoid arthritis patients. Despite showing efficacy in both collagen- and adjuvant-induced rodent models of arthritis, and being well tolerated in human arthritis patients in Phase I studies, it failed to show efficacy in human arthritis patients [84]. Further clinical development of apratastat was reported to be discontinued by Wyeth in 2006 [85]. INCB-7839 has been tested in a Phase I dose escalation study in healthy volunteers and a dose-dependent reduction in circulating levels of the HER2 extracellular domain was reported, which indicates efficacy as a TACE inhibitor *in vivo* [86]. This maintenance of high levels of HER2 at the cell surface may underlie the synergy observed between TACE inhibitors and suboptimal doses of trastuzumab *in vitro* and *in vivo* [81].

6. Conclusions

TACE-dependent shedding of cell surface proteins such as TNF- α and EGFR ligands plays an important role in development and disease. Whereas most of the clinical interest in TACE so far has centered on arthritis, a wave of preclinical studies in a variety of tumor model systems have provided strong evidence to support the contention that TACE is a new druggable target that could be used to inhibit pathogenic EGFR signaling in cancer. A number of effective TACE inhibitors are in existence, more are being developed and clinical trials are now getting underway to address this question.

7. Expert opinion

Inhibition of TACE offers another attractive therapeutic target in tumors dependent on EGFR signaling and trials are beginning this year to determine whether this strategy, which has shown considerable promise in preclinical studies, has value in the clinic. Although several of the existing TACE inhibitors have not proved effective in the treatment of arthritis, it must be remembered that arthritis is a complex multifactorial disease [87] so the failure of a single agent might not be unexpected. It is very encouraging that, where biomarkers of TACE inhibition have been assessed, several of these compounds are active at doses that are clinically achievable in patients. Thus, in principle, it is now possible to address the potential use of these compounds in cancer patients. The optimal use of TACE inhibitors in the oncology clinic is far from being determined, but, based on the preclinical data, it seems likely that they will find their most effective application in combination with existing targeted therapies. Certainly, in preclinical models, TACE inhibitors have been shown to act synergistically with existing chemotherapies [80].

Although the role of EGFR in several tumor types seems clearcut, there remains considerable debate about the importance of EGFR in breast cancer. In this field, most of

the clinical interest on the ErbB pathway has focused on ErbB2 (HER2), which is amplified and overexpressed in up to a third of breast cancers. Since this initial report [88], this receptor tyrosine kinase has been the target of extensive drug development efforts. The blocking antibody, trastuzumab, is now a cornerstone in the treatment of women tumors overexpressing this protein in both the adjuvant and metastatic settings. The extent to which breast tumors, or subsets of these tumors, may respond to EGFR inhibition remains largely an open question. Development of clinically effective EGFR inhibitors, such as gefitinib and erlotinib, lagged behind the development of trastuzumab. No Phase III study on the effect of these compounds in a substantial cohort of breast cancer patients has yet been reported and reports from Phase I and II studies in which some breast cancer patients were included have been mixed. Partial responses and maintenance of stable disease have been reported in some studies with gefitinib [89,90], whereas other trials reported no benefit [91,92]. It remains a possibility that gefitinib may have some benefit in subpopulations of patients with distinct clinical phenotypes, such as the basal subtype that the author has reported are enriched in TACE and TGF- α , or in patients in earlier stages of the disease (most of the trials were carried out in heavily pretreated patients). Interestingly, the dual EGFR/ErbB2 small-molecule inhibitor, lapatinib, has been used successfully in patients whose tumors have progressed on trastuzumab, which suggests that the EGFR may be playing a heretofore underappreciated role in this disease [32].

The failure of so many metalloprotease inhibitors, which appeared so promising in preclinical studies, caused a significant amount of consternation [93], which may fuel a reluctance on behalf of the major pharmaceutical companies to invest heavily again in agents that target this class of enzyme. It is noteworthy that most of the Phase III trials of MMP inhibitors in humans were performed on patients with advanced metastatic disease that had already proven refractory to standard chemotherapies. Accordingly, agents that are at best cytostatic might not fairly be expected to have a significant benefit in this clinical setting. Similarly, the ability of these agents to attenuate metastasis in animal models could not be, for obvious reasons, evaluated in patients who already had metastatic disease. It is also important to note that these trials were designed when knowledge of metalloprotease biology was in its infancy. When the preclinical and Phase I studies were being performed, only a small number of MMPs were known and their biology was poorly understood. The prevailing assumption of the time was that these proteases were important for basement membrane degradation during tumor progression. It has become clear that the 25 MMPs (not to mention the structurally related ADAM and ADAM-TS proteases) play significantly more diverse roles in human physiology [94] and that their inhibition, using broad-spectrum agents, would be expected to elicit significant pleiotropic effects.

Accordingly, the perspective that agents which very selectively target particular MMPs with high specificity while sparing those MMPs with important roles in tissue homeostasis may prove clinically useful is beginning to attract attention [95]. Nevertheless, these multiple failures were extremely disappointing and many companies have been reluctant to consider the possibility of metalloprotease inhibitors in oncology.

TACE inhibition in cancer offers a more clearly defined target. In the earlier trials, metalloproteases were targeted in the hope of suppressing clinically important yet hopelessly multifactorial processes, such as tumor cell migration and invasion. Strategies such as this offer little hope for rational patient selection to identify those who are most likely to benefit from the treatment. In contrast, targeting the EGFR pathway with TACE inhibitors offers a well-defined molecular target with many potential surrogate markers for efficacy, such as circulating levels of EGFR ligands or the phosphorylation (activation) status of the receptor itself or of the members of its downstream signaling network. Markers such as these have already begun to be used in trials of agents targeting the EGFR pathway [91]. It is as yet too early to know which factors might be predictors of TACE inhibitor sensitivity, but it is tempting to speculate that tumors that

express high levels of EGFR and one or more ligands, while lacking activating mutations in downstream proteins, such as Ras and Raf, would be a good place to start. It is also noteworthy that inhibitors of TACE/ADAM17 are often potent inhibitors of ADAM10. Together, suppression of TACE and ADAM10 should prevent the shedding of all known ErbB family ligands. Here, the protease inhibitor strategy may offer a considerable advantage over using single agents to target EGFR or ErbB2, as they should also prevent ligand-dependent activation of ErbB3, which has been recently associated with resistance to both gefitinib and trastuzumab [96,97].

In conclusion, we have come a long way from the 'boom and bust' of the first wave of MMP inhibitors and, hopefully, learned many important lessons along the way. We are now well into the era of targeted therapeutics and, although these too have had their teething problems, we now have a much better idea about how to best conduct appropriate trials of these agents [98]. Unlike many newly identified putative targets for cancer treatment, we are fortunate with TACE that our colleagues in rheumatology have done much of the 'heavy lifting' in terms of drug development and safety testing. More advanced human trials of TACE inhibitors in cancer are beginning this year and we await the outcome of these studies with considerable interest.

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