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EDITORIAL

Role of receptor tyrosine kinases in gastric cancer: New targets for a selective therapy

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Abstract

Receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor family participate in several steps of tumor formation including proliferation and metastatic spread. Several known RTKs are upregulated in gastric cancer being prime targets of a tailored therapy. Only preliminary data exist, however, on the use of the currently clinically available drugs such as trastuzumab, cetuximab, bevacizumab, gefitinib, erlotinib, and imatinib in the setting of gastric cancer. Preclinical data suggest a potential benefit of their use, especially in combination with "conventional" cytostatic therapy. This review summarizes the current knowledge about their use in cancer therapy as well as new approaches and drugs to optimize treatment success.

Key words: Gastric carcinoma; EGFR; Gefitinib; Trastuzumab; Cetuximab; Imatinib; Erlotinib; Bevacizumab

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INTRODUCTION

Gastric cancer is a common, but difficult to treat disease entity. Apart from potentially curative surgery, chemotherapy as well as radiochemotherapy may be applied, but do not cure the disease[1]. Thus, improvement of gastric cancer therapy will depend on novel therapeutic approaches. Receptor tyrosine kinases (RTKs) are a family of 56 proteins characterized by a transmembrane domain and a tyrosine kinase motif[2]. They function in cell signalling and transmit signals regulating growth, differentiation, adhesion, migration, and apoptosis[3]. Aberrant receptor tyrosine kinase activity was initially described in various epithelial cancers; nowadays, it is well accepted that receptor tyrosine kinases play an important role in almost all types of cancer[4,5]. The mutational activation and/or overexpression of receptor tyrosine kinases transforms cells and often plays a crucial role in the development of cancers.

For this reason, RTKs have become targets of immunological inhibitors, the best known being trastuzumab (Herceptin®, directed against the erb B2 receptor, also known as Her2/Neu oncogene) and cetuximab (Erbitux®, active against the epidermal growth factor receptor [EGFR]). In the case of EGFR, pharmacological inhibitors such as gefitinib (Iresssa®) directed at the kinase domain (EGFR-TKI) of the receptor have been developed; other pharmacological inhibitors of tyrosine kinases include imatinib (Gleevec®) which is licensed for the treatment of leukaemia and gastrointestinal stroma cell tumors (GIST).

As tailored concepts of cancer therapy evolve, RTKs offer prime targets for such an individualized approach to cancer treatment. This review examines the information available on this group of receptors, describes the most relevant subsets that have been found in case of gastric cancer, and summarizes data on the use of their inhibitors in clinical studies. In the end, we present new attempts to optimize the efficacy of already available compounds and promising new drug developments.

Receptor tyrosine kinases

RTKs are membrane bound proteins consisting of a ligand-binding domain at the extracellular surface, a single transmembrane segment, and a cytoplasmic part harboring the protein kinase activity. With the exception of the insulin receptor family of RTKs, all known RTKs form monomers in the cell membrane. Ligand-induced dimerization, resulting in autophosphorylation of their cytoplasmic domains, is the major mode of activation of RTKs.

The known 56 RTKs are divided into 21 families with similar structure and the potential of intrafamilial dimerization; their classification has been reviewed by Robinson *et al*[2]. The best known examples are the epidermal growth factor receptor family (erb B1 to B4), the different vascular endothelial growth factor receptor (VEGFR) subtypes, the

fibroblast growth factor (FGF) receptor family, and the platelet derived growth factor (PDGF) receptor family.

RTKs present in gastric cancer

We have recently analyzed the expression of RTK mRNAs in different human cancers, including 12 samples of gastric cancer[6]. The median of relative gene expression levels of the 56 known RTKs in these cancer specimens are illustrated in Figure 1. Our findings suggest that several RTKs, including those of the EGFR family, the FGFR family, and the different VEGFR subtypes are present in gastric cancer and thus offer potential targets for a selective therapy. In the following, we will summarize findings by others regarding the expression of the RTKs in gastric cancer with emphasis on those for which clinically established modalities of therapeutic intervention exist, namely the EGFR family and VEGF.

EGFR family – EGFR and erb B2

The EGF receptor/ligand system seems to be involved in the regulation of gastric mucosal proliferation and progression of gastric carcinoma. Increased EGFR binding has been observed in gastric carcinomas in comparison to adjacent normal gastric mucosa. Moreover, elevated EGFR levels have been found in gastric carcinoma with worse prognostic factors (T3/4, positive lymph-nodes, G3, diffuse-type). In univariate and multivariate analysis, EGFR levels are an independent indicator of poor prognosis[7,8]. A down-regulation of an inhibitor of EGFR activation, EGFR related protein (ERP) occurs in gastric cancer as well[9]. Changes in the constitutive phosphorylation and activity of tyrosine kinases such as EGFR may contribute to differences in cell adhesion and phenotype of gastric cancer cells[10]. Espinoza *et al* described that gastric adenocarcinomas potentially depend upon the TGFalpha-EGFR autocrine loop for growth and exhibit increased aggressiveness in the presence of aberrant p53[11].

The overexpression of the growth factor receptor oncogene erb B2 in gastric carcinomas was first described by Yonemura *et al* in 1991, who reported a 12% positive rate in 189 gastric cancers[12]. These findings were confirmed by many other studies, which provided further evidence that the highest rate of overexpression was found in patients with advanced disease, and that erb B2 altered expression can be considered as an independent predictor of outcome[13,14]. Ougolkov *et al*[15] found a more frequent overexpression of erb B2 in gastric cancers with concurrent liver metastasis than in those without, and concluded that activated erb B2 may be involved in the process of liver metastasis thus suggesting a role for erb B2 overexpression in identifying gastric cancer patients who are at high risk of developing liver metastasis. In contrast, activating mutations of the erb B2 tyrosine kinase seem to be rare events in gastric cancer[16].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is expressed in many gastric carcinoma cell lines and may play an important role in cell growth directly. VEGFR is virtually ubiquitous in human tumors and may promote tumor growth and metastasis by participating in both paracrine and autocrine pathways as higher levels of VEGFR have been correlated with more aggressive disease[17]. KDR is the main human receptor responsible for the VEGF activity in both physiological and pathological vascular development, and VEGF-KDR signalling pathway has been validated as a priority target for the development of anti-cancer therapy. A first report on 16 patients with early gastric cancer (pT1-T2, N0) suggested that expression of VEGF may indicate an increased risk of recurrence, making VEGF neutralization an interesting therapeutic target in these subjects[18].

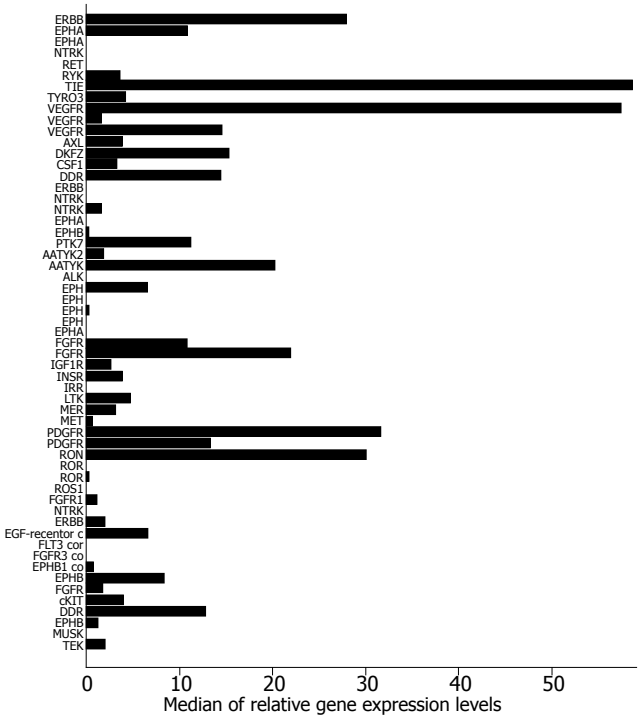

Figure 1 Median gene expression of receptor tyrosine kinases in 12 gastric cancer specimens.

Fibroblast growth factor receptor family

We have previously described an increased expression and autocrine stimulation of fibroblast growth factor (FGF) 2 mRNA in the course of gastric ulcer healing[19]; with respect to gastric cancer, the secretion of growth factors of the FGF family by fibroblasts stimulates proliferation of cancer cells of scirrhous gastric cancer[20]. The expression of FGF2 mRNA (formerly known as basic fibroblast growth factor, bFGF) in gastric cancer specimens is associated with higher microvascular density, tumor progression and a worse prognosis[21]. An orally active inhibitor of FGF-receptor autophosphorylation has been successfully tested in animal models of gastric cancer; further studies are awaited[22].

Platelet derived growth factor receptor

Already more than a decade ago, the expression of platelet derived growth factor (PDGF) and its receptor mRNAs were linked to gastric cancer growth[23]. Later on, the expression of PDGF-A mRNA in biopsy samples of gastric cancer specimens was determined to be a preoperative prognostic marker associated with shorter survival[24]. As

imatinib mesylate as an inhibitor of the PDGFR tyrosine kinase became available, its effectiveness against gastric cancer cells was assessed *in vitro* and *in vivo*. Unfortunately, the agent itself exhibited no cytostatic effect of relevance; yet in combination, imatinib mesylate might serve as an effective chemosensitizer of antitumor drugs, such as 5-FU and paclitaxel for gastric carcinoma, targeting the PDGF/PDGFR-signalling pathway of tumor cells and stromal cells in disease progression and angiogenesis[25].

Leflunomide is a small molecule inhibitor of PDGFR-mediated phosphorylation and inhibits PDGF-mediated cell signalling[26]; it is converted to its main metabolite, SU0020, which interferes with de novo pyrimidine synthesis. At this time, it is not clear whether the mechanism of action of this drug in humans is due to inhibition of PDGF-dependent signalling, inhibition of pyrimidine synthesis, or a combination of both[27-29]. A multi-institutional phase II study in hormone refractory prostate cancer patients with leflunomide found partial responses in 1 of 19 patients, a prostate-specific antigen decline greater than 50% in 3 of 39 patients, and improvement in pain[27].

Hepatocyte growth factor receptor / c-MET

Soman *et al* were able to identify TPR-MET fusion as an event of gastric cancer pathogenesis in certain cases[30]; in addition, c-MET activation seemed to occur[31]. Preliminary data raised in animal models of gastric carcinoma suggest that adenoviral-mediated gene transduction of an HGF antagonist (NK4) results in suppression of tumor growth, invasion, angiogenesis, and metastasis[32,33]. The use of this construct was able to overcome resistance against gefitinib in an animal model of scirrhous gastric cancer[34], yet clinical data is lacking.

USE OF RTK INHIBITORS IN GASTRIC CANCER

Available RTK inhibitors

The inhibitors of tyrosine kinases currently approved by the United States Food and Drug Administration (FDA) for the use in different human malignancies are summarized in Table 1. Substances under development are described in the paragraphs below. If treatment results of RTKIs are to be evaluated, one has to keep in mind that inhibition of a growth factor receptor does not typically aim at killing the cell like "conventional" cytostatics, but stoping proliferation. Therefore, standard assessments of tumor response like complete response, partial response, and stable disease may not reflect the "true" efficacy of such a regime.

Table 1 FDA-licensed tyrosine kinase inhibitors, their targets, modes of action, and clinical use

Agent	Target	Mode of action	Established clinical application
Imatinib mesylate	bcr-abl; PDGFR, c-kit	Kinase inhibitor	CML, GIST, hypereosinophilic syndrome
Gefitinib	erbB1/EGFR	Kinase inhibitor	NSCLC
Erlotinib	erbB1/EGFR	Kinase inhibitor	NSCLC
Cetuximab	erbB1/EGFR	Blocking antibody	Colorectal cancer
Trastuzumab	erbB2/HER	Blocking antibody	Breast cancer
Bevacizumab	VEGF	Neutralizing antibody	Colorectal cancer

Immunological agents

VEGF - Bevacizumab In contrast to the other immunological agents which attach directly to the receptor of the tyrosine kinase, the currently available antibody bevacizumab interrupts the signal cascade by neutralizing the ligand. In a study comprising over 800 patients with metastatic colorectal cancer, the addition of bevacizumab to irinotecan, 5-fluorouracil, and leucovorin as first-line therapy led to a significant increase in survival time[35]. Recent studies concerning the neutralizing antibody bevacizumab, and the small molecule tyrosine kinase inhibitor SU5416, demonstrated that, while unlikely to be effective as monotherapy, incorporation of VEGFR blockade into cytotoxic regimens may increase overall response rates in solid tumors. However, incorporation may also introduce new toxicities, including thromboembolic complications and bleeding[36-38]. Gastrointestinal bleeding, epistaxis and thrombotic events have been seen in 10.3%, 50% and 19.1%, respectively, of the patients receiving bevacizumab alone[39]; these side effects should be taken into consideration when deciding about bevacizumab therapy. There is a phase II trial of bevacizumab, irinotecan, and cisplatin in metastatic or unresectable gastric cancer (National Cancer Institute [NCI] protocol 6447) currently active, of which only the high rate of thromboembolic events in six (25%) of 24 patients (95% CI, 11% to 45%) has recently been reported[40]. However, the incidence of thromboembolic events was not significantly different when compared to a similar irinotecan-based protocol not containing bevacizumab, and there was no difference in the pattern of venous thromboses.

A small molecule inhibitor of VEGFR2 (Vatalanib; PTK787/ZK 222584) has been developed; preliminary data on its use in colorectal cancer exist[41]. With respect to gastric cancer, a Japanese group reported the effects of another VEGFR tyrosine kinase inhibitor (SU6668) in a mouse model of gastric cancer. SU6668 did not directly alter the growth rate of the cancer cells, but inhibited tumor angiogenesis, resulting in the inhibition of tumor dissemination in the peritoneum[42].

Erb B2 / HER-neu – Trastuzumab When considering how EGFR/erb B-targeted therapeutics function, it is important to mention that, in contrast to the small molecule kinase inhibitors, antibodies targeting EGFR and erb B2 have the inherent ability to recruit immune effector cells such as macrophages and monocytes to the tumor through binding of the antibody constant Fc domain to specific receptors on these cells. In xenograft models at least, this mechanism is relevant with the anti-tumor activity of erb B2-targeted trastuzumab[43]. Whether this mechanism has a role in clinical efficacy in cancer patients remains unproven[44]. Trastuzumab (Herceptin) is a humanized monoclonal antibody, which was approved by the United States FDA in 1998 for the treatment of advanced breast cancer. This was the first approval of a monoclonal antibody for use in solid tumor therapy[45]. Recently, it was

investigated whether trastuzumab could affect the growth of HER/neu-overexpressing gastric cancer cells based on the antibody-dependent cell-mediated cytotoxicity (ADCC). It has been demonstrated that these cells could be killed by trastuzumab-mediated ADCC and this correlated with the degree of HER-2/neu expression[46]. However, the trastuzumab-mediated ADCC was significantly impaired when tested in peripheral blood mononuclear cells from patients with advanced disease as compared to those with early disease. Moreover, natural killer cells purified from patients with advanced disease showed less trastuzumab-mediated ADCC in comparison to those from healthy donors. Consequently it has also been postulated that some treatment modalities, such as those involving the use of interleukin 2, could contribute to reverse NK dysfunction, which may be necessary for successful trastuzumab treatment of gastric cancer. Funato *et al*[47] used MKN-7 and KATO-III gastric cancer cells, which express the erb B2 oncogene, to study the mechanism of resistance to cisplatin. They found that erb B2 expression in gastric cancer is related to cisplatin sensitivity, and that anti-erb B2 antisense oligonucleotides could induce increased sensitivity to this drug. Experimental therapy utilizing a different anti-HER mouse-human chimeric monoclonal antibody named CH401 documented efficacy in an *in vivo* model of gastric cancer, yet clinical data are lacking[45].

Epidermal growth factor receptor (EGFR) – Cetuximab

Cetuximab has been approved by the FDA for use in colorectal cancer; cetuximab and other molecules inhibiting the EGFR pathway in colorectal cancer have been recently reviewed[48,49]. One large trial of cetuximab in colorectal cancer comprising 329 patients with irinotecan-refractory metastatic cancer indicated a delayed median time to progression by combining cetuximab with irinotecan[50]. A known adverse characteristic of anti-EGFR therapy is an acne-like skin rash associated with treatment response, which was observed in about three out of four patients[51]. In contrast to gefitinib, the response of cancer cells to cetuximab occurs independent of the mutational status of the EGFR[52,53]. Unfortunately, there are no relevant clinical data available on the use of cetuximab in patients suffering from gastric cancer. As cetuximab is a chimeric antibody which may cause immunological reactions, humanized anti-EGFR antibodies have been developed, one being matuzumab, which currently undergoes phase II trials including studies in patients suffering from gastric cancer[54].

Pharmacological agents – small molecule tyrosine kinase inhibitors

Imatinib: Imatinib is an established and licensed treatment modality in gastrointestinal stroma cell tumors (GIST)[55], but with respect to gastric cancer, only limited data about its use exist. A single study in an animal model of gastric cancer suggested no independent activity of imatinib, but proved an effective chemo- sensitization of antitumor drugs, such as 5-FU and paclitaxel for gastric carcinoma, targeting the PDGF/PDGFR-signalling pathway of tumor cells and stromal cells in disease progression and angiogenesis[25].

EGFR-inhibitors–Erlotinib and Gefitinib

The first representative of this drug class to be approved for cancer therapy was gefitinib (Iressa®) in the third line treatment of non-small-cell lung cancer[56]. A subsequent phase III trial (Iressa Survival Evaluation in Lung cancer, ISEL) failed to demonstrate survival advantage for those patients when compared with placebo[57]. Furthermore, in other malignancies such as gastric carcinoma, preliminary data indicate that treatment efficacy with this regimen is limited as well[58]. A phase II trial to investigate the efficacy, tolerability and pharmacokinetics of gefitinib in pretreated patients with metastatic gastric carcinoma included 75 subjects who were randomised to receive 250 or 500 mg/d gefitinib orally. The authors found that gefitinib monotherapy was generally well tolerated in pretreated patients with gastric metastatic adenocarcinoma, with disease control achieved in 18.3% of cases analyzed. The most common drug-related adverse events were diarrhea, rash and anorexia. The only dose-related adverse events were rash (25.0% at 250 mg/d *vs* 44.7% at 500 mg/d) and anorexia (8.3% at 250 mg/d *vs* 15.8% at 500 mg/d). Rojo *et al*[59]evaluated immunohistochemically the percentage of tumor cells expressing EGFR, pEGFR (the activated phosphorylated form), pMAPK, pAkt (phosphorylated Ser473) and Ki67, before and after treatment with gefinitib. Prior to treatment EGFR expression was found in 62.5% of tumors, whereas pEGFR levels were significantly reduced after the treatment. However, a decreased proliferation was observed only in those tumors with low levels of pAkt, suggesting a role for the PI3k-Akt pathway in gefinitib resistance. Recent studies suggested that clinical response to gefitinib in lung cancer depends on the presence of somatic mutations of the EGF receptor in the tumor which enhance the responsiveness of the receptor to EGF ligand and increase its sensitivity to inhibition by gefitinib[60-62]. In the case of gastric cancer, no such mutations are known. Data on the use of erlotinib (Tarceva®; OSI-744) in gastric cancer is limited to a single study of 70 patients having either gastric cancer (n = 26) or gastroesophageal junction cancer (GEJC) (n = 44). No patient in the gastric cancer cohort presented an objective response, but five patients in the GEJC cohort did so, one being a complete response. An overall response rate was 11%[63]. The best known therapeutics targeting members of the EGFR family which are currently available or under investigation are summarized in Table 2.

Attempts to optimize treatment efficacy of RTKIs

As resistance against single agents may arise and tumor survival may rely on more than one growth factor pathway, several attempts have been made to optimize treatment efficacy, most of them still being in preclinical testing. The following options exist: (1) Combination of inhibitors of the same pathway (e.g. gefitinib and cetuximab) in order to further enhance signal abrogation; (2) To combine inhibitors of different RTK pathways or apply non-selective inhibitors of several pathways in case the cancer cell loses its reliance on one specific pathway; (3) To select cells dependent on growth factor stimulation by use of other cytotoxic drugs or radiation and subsequently eliminate these by an immunological or pharmacological

RTKI.

Enhanced abrogation of one RTK pathway by combining different inhibitors

In 2004, two groups described the use of cetuximab combined with gefitinib and erlotinib as enhanced abrogation of the EGFR signal cascade in *in vitro* as well as *in vivo* models. Matar *et al*[64] utilized an EGFR-dependent human tumor xenograft model and found a synergistic effect on cell proliferation and superior inhibition of EGFR-dependent signalling and induction of apoptosis. Even suboptimal doses of gefitinib and cetuximab given together resulted in a complete and permanent regression of large tumors. In the combination-treated tumors, there was a superior inhibition of EGFR, mitogen-activated protein kinase, and Akt phosphorylation, as well as greater inhibition of cell proliferation and vascularization and enhanced apoptosis. Using cDNA arrays, 59 genes could be identified that were coregulated and 45 genes differentially regulated, including genes related to cell proliferation and differentiation, transcription, DNA synthesis and repair, angiogenesis, signalling molecules, cytoskeleton organization, and tumor invasion and metastasis. Huang *et al*[65] reported similar findings in head and neck tumors and in a model of lung cancer; they observed that gefitinib and erlotinib retained the capacity to inhibit tumor cell growth in case of cetuximab resistance. Again, the combination of antibody and kinase inhibitor resulted in more profound tumor regression and regrowth delay.

Another group applied trastuzumab and an inhibitor of EGFR and erb B2 tyrosine kinases, lapatinib, in erb B2-overexpressing breast cancer cell lines. Only in combination, treatment resulted in a markedly downregulated survivin protein expression and enhanced tumor cell apoptosis thus suggesting a potential improvement in clinical response[66]. But not all findings support the idea of combining inhibitors of the same pathway: in an *in vitro* model using two human epidermoid cell lines the combination of cetuximab and gefitinib demonstrated antagonistic effects. Administration of either drug alone led to a diminution in EGFR levels, while their combination increased the cellular expression of EGFR. These findings suggest that new and tempting treatment strategies on the EGFR target consisting in a double hit with a monoclonal antibody and a TKI must be considered with caution[67]. Data with regard to gastric cancer are currently lacking.

Inhibition of several RTK pathways: combination of different substances, non-selective inhibitors

Cancers possessing complex kinase profiles may respond better to a multimodal therapy tackling several pathways; and such regime may simultaneously reduce the emergence of resistance. Therefore a combination of different agents or a single "unspecific" inhibitor of several pathways may offer advantages over inhibition of a single pathway.

Combinations of different agents: As there is currently no single known inhibitor of all relevant receptor tyrosine kinases, combination of the well evaluated agents offers a straightforward concept of therapy, taking into account that in most cancer therapies different substances are being combined. First data were raised in phase I or II breast cancer trials by combining trastuzumab with small molecule inhibitors of EGFR such as gefitinib or erlotinib indicating that this combination provided a well tolerated targeted therapy with preliminary evidence of antitumor activity[68,69]. As activation of the EGF receptor may induce vessel formation by cellular liberation of VEGF, approaches blocking both pathways possess certain attractiveness. In conditions of limiting VEGF, EGF plays an important role in endothelial cell proliferation, survival, and sprouting of small vessels. Animal data suggest that combined inhibition of EGFR and VEGFR pathways may produce synergistic results and that resistance to EGFR inhibition may be overcome by inhibition of VEGFR tyrosine kinase[70,71]. In an *in vivo* model, the combination of anti-VEGF-R and anti-EGF-R therapies was effective in inhibiting gastric cancer growth whereas the decrease in tumor growth in mice treated with DC101 (an anti-VEGF-R antibody) or cetuximab alone did not reach statistical significance[72]. As mentioned above, the addition of an inhibitor of the hepatocyte growth factor was able to overcome resistance against gefitinib resulting from the interaction of stromal and cancer cells in an animal model of scirrhous gastric cancer[34].

Inhibitors not selective for a single pathway: Agents targeting multiple RTKs are currently undergoing preclinical testing or phase I studies. These include AEE788 (directed against EGFR, erbB2, VEGFR2; Novartis), BAY 43-9006 (Sorafenib; Raf kinase, VEGFR2, PDGF-R beta; Bayer), SU11248 (PDGF-R, VEGFR, c-kit-R, FLT3-R; SUGEN), and ZD6474 (VEGFR and EGFR, AstraZeneca), all designed to inhibit multiple mechanisms of tumor growth in addition to conventional chemotherapy[73-76]. Similar to the combination of anti-EGFR and anti-VEGFR antibodies mentioned above, McCarthy *et al*[77] tested the inhibitor of both tyrosine kinases, ZD6474, in an orthotopic model of gastric cancer. The agent led to marked

Table 2 Therapeutics targeting the EGFR family

Agent	Type	Target	Status
Trastuzumab	Humanized mAb	erbB2	Approved for breast cancer
Pertuzumab	Humanized mAb	erbB2	Phase II trials
Cetuximab	Chimeric mAb	EGFR	Approved for colorectal cancer
Matuzumab	Humanized mAb	EGFR	Phase II trials
Panitumab	Humanized mAb	EGFR	Trials ongoing
Gefitinib	TKI	EGFR	Approved for NSCLC
Erlotinib	TKI	EGFR	Approved for NSCLC
Lapatinib	TKI	EGFR/erbB2	Phase III trial / breast cancer
AEE788	TKI	EGFR/ erbB2/ VEGFR	Phase I trials
CI-1033	Irreversible TKI	EGFR/erbB2	Phase II trials
EKB-569	Irreversible TKI	EGFR/erbB2	Phase II trials
EXEL 7647 /TKI EXEL 0999		EGFR/ erbB2/ VEGFR	Phase I trials

(incomplete list; modified according to ref. 44)

inhibition of tumor growth, tumor cell proliferation, and decrease in microvessel density. The authors concluded that therapies such as ZD6474 that target two distinct aspects of tumor growth, angiogenesis and tumor cell proliferation, warrant further investigation.

Combination of standard chemotherapeutic agents and radiation with inhibition of RTKs

"Conventional" chemotherapy and RTKIs: The combination of antibody based therapies with conventional therapy has become a standard procedure in many cancers. For example, in colorectal cancer, cetuximab or bevacizumab is typically combined with irinotecan-based regimes[35,50]. One of the first reports on the use of an RTKI, trastuzumab, in breast cancer documented the inhibition of DNA repair subsequent to DNA damage by cisplatin by an antibody to Her2/Neu. Therapy with this antibody led to a 35%-40% reduction in repair of cisplatin-DNA adducts after cisplatin exposure and, as a result, promoted drug-induced killing in target cells[78]. Another study using human tumor xenografts found that gefitinib caused growth inhibition of tumors and enhancement of the activity of a number of cytotoxic drugs, but neither was dependent on high levels of EGFR expression[79]. In an animal model of pancreatic cancer, inhibiting phosphorylation of EGFR, VEGFR, and PDGFR by appropriate RTKIs in combination with gemcitabine enhanced the efficacy of gemcitabine alone, resulting in inhibition of experimental human pancreatic cancer growth and significant prolongation of survival[80]. Similar results were obtained in a model of estrogen receptor-positive breast cancer in which successful cooperation of the dual erb B1/B2 inhibitor lapatinib with tamoxifen was evidenced[81]. Yet there are pitfalls of combinatorial therapy: the combination of two substances may not always be beneficial; for example, the combination of tamoxifen and trastuzumab in estrogen-receptor positive breast cancer may be less effective than either substance alone possibly due to an increase in erb B2 signalling pathways that occurs when tamoxifen is added to trastuzumab[82]. In case of gefitinib, addition of this RTKI to platin-based chemotherapy in non-small cell lung cancer (INTACT 2 trial) showed no added benefit in survival, TTP, or RR compared with standard chemotherapy alone. This large, placebo-controlled trial confirmed the favorable gefitinib safety profile observed in phase I and II monotherapy trials[83]. Currently only data from animal or *in vitro* testing exist with respect to gastric cancer; Park *et al*[84] investigated the effect of gefitinib combined with oxaliplatin, 5-fluorouracil, or paclitaxel in a gastric cancer cell line, SNU-1. This study demonstrated the antitumor activity and a significant cell cycle arresting effect induced by gefitinib in SNU-1 human gastric carcinoma cells, and its synergistic interaction with oxaliplatin and paclitaxel. There are preliminary data on a multicenter phase II study of irinotecan, cisplatin, and bevacizumab in gastric or gastroesophageal adenocarcinoma which indicate an excellent disease control rate of 13/15 cases; in a subset of ten patients with measurable disease who had received at least two cycles of therapy, 5 partial responses, 4 minor responses (15%-29% reduction) and 1 stable disease were observed[85]. Other, less toxic substances have been assessed as well. Thus it has previously been documented that gastric tissue exposed to acetylsalicylic acid (ASA) expresses high levels of EGFR[86,87]. A positive loop regulation between COX and erbB2[88] as well as EGFR[89] has been postulated. Since nonsteroidal anti-inflammatory drugs (NSAIDs) might be a tool of carcinoma prevention in the gastrointestinal tract[90] we investigated the mechanisms of a potential synergism of simultaneous cyclooxygenase (COX)- and EGFR-inhibition. It has previously been described that simultaneous administration of COX- and EGFR-inhibitors exerts tumor preventive effects in nude mice[91]. A combination of these two substances with a protein kinase A (PKA) antisense oligonucleotide was able to eliminate tumors in more than half of the animals treated[92]. Preliminary data from our group indicate that acetylsalicylic acid may modulate the expression and activation of EGFR in gastric cancer cells rendering them more susceptible to gefitinib treatment[93].

Radiation therapy and RTKIs: Radiation causes cell death by induction of cellular injury which may rely on subsequent growth factor/receptor tyrosine kinase activation for repair, the combination of radiation and RTKIs seems to be obvious. Currently, however, only data with respect to experimental therapy, especially in animal models, exist. E.g., She *et al*[94] examined the effect of addition of gefitinib to radiation therapy in a nude mouse model of different cancers, including lung and breast cancer. Gefitinib significantly enhanced the antitumor action of radiation therapy against the test tumors without significant adverse effects, increasing the therapeutic selectivity of ionizing radiation in these model systems. In a similar model, PTK787/ZK222584, a specific inhibitor of VEGFR tyrosine kinases, was tested. Tumors vascularized by radiation-damaged vessels responded to PTK787/ZK222584 with longer latency and slower growth rate than controls, and a trend toward further increase in necrosis, indicating that irradiated tumor vessels are more susceptible to VEGFR inhibition than unirradiated vessels[95].

CONCLUSION

Receptor tyrosine kinases participate in several steps of tumor formation including proliferation and metastasis formation. As several of them are upregulated in gastric cancer, they offer potential prime targets for a tailored therapy. Unfortunately, only preliminary data exist on the use of the currently clinically available drugs such as trastuzumab, cetuximab, bevacizumab, gefitinib, erlotinib, and imatinib in the setting of gastric cancer. However, phase II trials are underway to examine the potential of these drugs in adenocarcinoma of the stomach. As RTK inhibitors with a broad range are being developed, the potential usefulness of this drug class is most likely to further increase as preclinical data in models of gastric cancer already indicate their effectiveness. The approach of combining RTKIs with "conventional" means of tumor therapy such as cytostatics and radiation therapy is most likely to find its way into clinical application in near future. RTKIs alone typically only inhibit tumor growth and do not aim at killing the cancer cell, but they might prove to

be an effective chemosensitizer of other antitumor drugs as they are able to block the process of cellular restitution following injury caused by radiation or "conventional" cytostatics. Different trial designs may be necessary given that some RTKIs might only work in small subsets of patients (for example, the fate of gefitinib in non-small cell lung cancer) and often improve patients' condition without leading to an objective response. Genomic or proteomic analysis of aberrations in individual patients may be necessary to decide about a tailored therapy.

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