

Membrane Receptor and Antiangiogenic Targeted Therapies in the Treatment of Cancer

Antonio Jimeno* and Hernán Cortés-Funes

Medial Oncology Division, University Hospital 12 de Octubre, Madrid, Spain

Abstract: The rapidly expanding knowledge of the pathogenesis of a variety of forms of cancer at the molecular level is now providing new targets for drug discovery and development. This has enabled us to successfully develop rationally designed therapies for cancer patients, and the results of their clinical evaluation are now becoming available. The optimal clinical development of target-based anticancer drugs will require fundamental changes to the way trials are designed, outcome is evaluated, and patients are selected to receive therapy. A thorough knowledge of what is accomplished so far is the cornerstone to optimally develop and implement these new strategies.

Keywords: Targeted therapy, EGFR, HER2, VEGFR.

1. INTRODUCTION: TARGETED THERAPIES

Despite improvements in survival rates, cancer remains the second leading cause of death in the US [1]. Unfortunately, the majority of patients are treated with a palliative intent, and therefore quality of life and tolerability issues are of paramount importance. Traditional chemotherapy agents are designed to block cell division, and therefore can be toxic to healthy cells as well as cancer cells. Many cytotoxic agents have a relatively narrow therapeutic margin, and in many patients with incurable disease, the odds for suffering from treatment-related adverse effects are superior to the probability of obtaining a clinical benefit. Targeting specific pathways to stop cancer growth can be less toxic to normal cells, and thus improve tolerability. The rapidly expanding knowledge of the pathogenesis of a variety of forms of cancer at the molecular level is now providing new targets for drug discovery and development. Anticancer drug discovery has shifted from an empiric random screening approach to a more rational and mechanistic, target-based approach, where specific abnormalities in cell functioning are modulated in a classical drug-receptor fashion (Table 1).

The optimal clinical development of target-based anticancer drugs will require fundamental changes to the way trials are designed, outcome is evaluated, and patients are selected to receive therapy. A thorough knowledge of what is accomplished so far is the cornerstone to optimally develop and implement these new strategies.

2. THE HER FAMILY OF MEMBRANE RECEPTORS

The HER family of membrane receptors is one of the most exciting targets currently under evaluation. This family

is composed of four members: HER1 (also known as the epidermal growth factor receptor, EGFR), HER2 (also termed ErbB2 or HER2/*neu*), HER3 (also termed ErbB3), and HER4 (also termed ErbB4). These receptors share the same molecular structure with an extracellular ligand binding domain, a short transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity (excepting the HER3) [2]. The binding of different ligands to the extracellular domain initiates a signal transduction cascade that can influence many aspects of tumour cell biology, including cell proliferation, apoptosis, adhesion, migration, and differentiation [2-4]. Ligand binding induces EGFR homodimerization, as well as heterodimerization with other types of HER proteins. HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner for other members of the HER family [5]. This crosstalk between membrane receptors may be relevant for the development of antireceptor therapeutics as it is a potential source of bypassing therapeutic interventions. As a matter of fact, HER1/HER1 homodimers are unstable, whereas HER1/HER2 heterodimers are stable, and recycle rapidly to the cell surface [6]. Therefore, HER2 overexpression can enhance EGFR signalling, increasing output to downstream pathways. In addition, high levels of HER2 expression alter the ability of small molecules with TK inhibitor activity to efficiently block EGFR phosphorylation [7]. HER3 and HER4 are variably expressed in breast and other cancers.

The HER family of receptors was proposed as a rational target for drug development more than 20 years ago [8, 9]. Consequently, therapeutic agents targeting this family have been developed. Over the last few years, an increasing number of compounds directed against the EGFR and HER2 have entered clinical development and are currently in clinical trials. In the next few paragraphs we summarize the key clinical and translational research aspects in the development of these drugs.

2.1. Targeting the EGFR (HER1)

The EGFR is overexpressed in many different solid human tumours, and this overexpression has been associated with advanced stages of disease, resistance to conventional

*Address correspondence to this author at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 162A, 1650 Orleans Street, Baltimore, MD, 21231-1000, USA; Tel: 410 5025835; Fax: 410 6149006; E-mail: ajimeno1@jhmi.edu

Table 1. Differences Between Empirical and Targeted Drug Development

	Empirical	Biologically directed
Discovery	Based on random cell screening	Based on receptors
Mechanism of action	Undetermined by screening	Basis of selection
Pharmacologic effect	Cytotoxic (irreversible)	Cytostatic (reversible or irreversible)
Specificity	None (toxic)	Selective (less toxic)
Dose and administration	Cyclic (following MTD)	Continuous/Cyclic

MTD: Maximum tolerated dose.

treatments, and poor prognosis [3, 10, 11]. Blockade of the EGFR was shown to stop cell proliferation both in *in vitro* and *in vivo* cancer models [8, 9, 12]. Numerous classes of drugs, which target EGFR are under development, but two strategies have been more extensively explored in clinical trials: the use of monoclonal antibodies (MAbs) directed against the external domain of the receptor, and the use of small molecules that compete with adenosine triphosphate (ATP) for binding to the receptor's kinase pocket, thus blocking receptor activation, also known as TK inhibitors (TKI).

2.1.1. Monoclonal Antibodies (MAbs) Targeted Against the EGFR

Blocking altered biological pathways with MAbs is one of the most successful therapeutic strategies currently under evaluation in cancer research, and the EGFR is one of the targets against which more MAbs are being developed.

Cetuximab (IMC-C225; Im-Clone Systems, New York, NY) is a quimeric mouse-human MAb that binds the EGFR in its extracellular domain, and blocks EGF-induced autophosphorylation of the EGFR cell lines *in vitro* [13], induces dimerization and downregulation of the EGFR [14], perturbs cell cycle progression by inducing a G₁ arrest through an increase in the protein levels of the p27^{kip1} inhibitor of cyclin-dependent kinases [15], and inhibits tumour-induced angiogenesis [16]. Cetuximab evidenced preclinical activity *in vitro* and *in vivo*, as single agent and in combination with cytotoxic agents and radiotherapy in a wide range of human cancer cell lines, including colorectal, pancreatic, prostate, breast, head and neck, glioma and ovarian cancer.

In phase I studies doses ranking from 5 to 400 mg/m² were explored without reaching a maximum tolerated dose (MTD). Pharmacokinetic analyses showed a non-linear behaviour, with saturation of drug clearance at doses over 200 mg/m², and therefore the dose regimen selected for phase II-III trials was a loading dose of 400 mg/m² followed by a weekly maintenance dose of 250 mg/m². Phase I trials revealed a favourable tolerability, with the most significant toxicity reported being an acneiform rash and folliculitis involving the face and upper chest, which occurs in 80% of patients [17-19]. Hypersensitivity reactions have been reported, some of them occurring within minutes of the first infusion, but they are uncommon and rarely life-threatening. Other adverse effects include asthenia, fever, and alteration in liver function tests.

A number of studies have evaluated cetuximab alone or in combination with radiotherapy or chemotherapy in patients with squamous head and neck, renal, pancreatic, colorectal, and non-small cell lung cancer (NSCLC). Of special relevance is the study by Saltz *et al.*, that reported the results of a phase II trial of irinotecan in combination with cetuximab in 120 patients with metastatic EGFR-positive colorectal carcinoma previously treated with irinotecan [20]. The objective response rate was 17%, and an additional 31% of the patients had minor responses or stable disease. Interestingly, response rate was significantly higher among patients that experienced skin rash (29%) than among those who did not (3%), a finding documented in other cetuximab studies across multiple malignancies [21]. The results of a phase II trial that compared the objective confirmed response rate of the combination of cetuximab plus irinotecan, or of cetuximab as a single agent in patients with EGFR-positive, irinotecan-refractory colorectal cancer patients have been recently communicated [22]. Response rate and time to progression were longer in the combined therapy arm, and cetuximab is currently approved for use in patients with EGFR-positive, irinotecan-refractory colorectal cancer patients. In a phase III study that randomised patients with squamous carcinoma of the head and neck (SCCHN) to cisplatin plus cetuximab or placebo, an increase in the response rate of subjects receiving cetuximab was documented (23% vs. 9%), although no differences in progression-free or overall survival were noted [23]. Preliminary data from a phase III study of radiation therapy with or without cetuximab in patients with advanced SCCHN has demonstrated a significant prolongation of overall survival in patients treated with the combined treatment (28 vs. 52 months, p=0.02) [24]. Cetuximab monotherapy has shown modest activity in refractory NSCLC [25], and the addition of cetuximab to cisplatin plus vinorelbine versus chemotherapy alone as first-line treatment of patients with NSCLC has shown evidence suggesting an increase in activity, although more mature reports are awaited [26].

ABX-EGF is a high-affinity, fully human MAb that binds the EGFR in its extracellular domain, and blocks the binding of both EGF and TGF- α to various EGFR-expressing human carcinoma cell lines, and inhibits EGF-dependent tumour cell activation, including EGFR tyrosine phosphorylation and cell proliferation [27]. Fully humanization prevents the development of antibodies against murine epitopes, a potential limitation of MAbs that could result in decreased efficacy. ABX-EGF has evidenced

preclinical activity *in vitro* and *in vivo*, as single agent and in combination with cytotoxic agents in a wide range of human cancer cell lines, including pancreatic, prostate, breast, head and neck, and renal human cell lines [28]. Based on these preclinical studies a biomathematical model was developed to predict antitumour efficacy in patients, and maximum antitumour activity was calculated for maintenance doses between 1 mg/kg and 3 mg/kg weekly. Initial clinical trials of ABX-EGF in heavily pretreated patients have reported encouraging results. A phase I trial was conducted with a weekly schedule with doses ranging from 0.01 to 2.5 mg/kg weekly for four consecutive weeks and every other week thereafter. In this study, treatment with ABX-EGF was well tolerated and no dose limiting toxicities were reported [29]. Biological activity, evidenced by reversible acneiform skin rash, was observed at the 1.0 mg/kg dose level. A phase I/II disease-directed study of ABX-EGF monotherapy in 58 patients with metastatic renal carcinoma has documented two partial responses, and 18 more minor responses or disease stabilization [30], and in another study in 148 patients with metastatic EGFR-positive colorectal carcinoma a response rate of 10% has been documented [31].

EMD72000 is a humanized MAb directed at the EGFR that has shown potent antitumour activity in preclinical studies [32, 33]. A significant difference with the abovementioned EGFR-targeted monoclonal antibodies is a longer half-life time, a feature that has prompted its evaluation every two and three weeks [34]. Clinical activity was documented in subjects with colorectal and renal cancer. This study included pharmacodynamic assessment and correlative studies, evidencing that EMD72000 induced a complete inhibition of pEGFR and pMAPK with an increase in p27 in skin biopsies in all patients, whereas pAKT was only inhibited in responding patients. An expanded phase of the prior study has shown dose-dependent inhibition of downstream pathways in surrogate tissues, supporting an optimal biological dose-seeking approach [35]. A phase I exploring six different doses in heavily pretreated subjects with gastrointestinal cancers showed preliminary evidence of metabolic response by PET and antitumour activity by CT and MRI [36].

hR3 is a genetically engineered humanized MAb targeted against the EGFR. The antibody exhibits potent *in vitro* and *in vivo* antitumour effect on EGFR overexpressing cell lines, as well as antiangiogenic activity [37]. Preliminary data in cancer patients has evidenced a favourable toxicity profile [38, 39], and several phase II trials are now underway to

evaluate the efficacy of hR3 in the treatment of advanced cancer patients.

2.1.2. Tyrosine Kinase Inhibitors (TKIs) Targeted Against the EGFR

A large number of TKIs are currently being evaluated. They can be classified according to their selectivity (monofunctional agents with HER1-specificity, as opposed to multifunctional agents, with panHER-specificity), and according to the reversibility of their interaction with their target (reversible or irreversible inhibitors) (Table 2).

Gefitinib (ZD1839; AstraZeneca, Wilmington, DE) is an orally active, low molecular weight, synthetic quinazoline. Gefitinib reversibly and selectively targets the EGFR and blocks signal transduction processes implicated in the proliferation and survival of cancer cells with minimal activity against other tyrosine kinases and serine/threonine kinases. Gefitinib prevents autophosphorylation of EGFR, resulting in the inhibition of downstream signalling pathways [40-42]. In animal studies, gefitinib has shown antitumour activity against a wide range of human tumour xenografts [43].

Phase I clinical trials of gefitinib showed a good toxicity profile, mostly consisting in skin toxicity and diarrhoea, and DLTs were observed at doses well above that at which antitumour activity was seen [44-46]. Phase I studies in combination with conventional chemotherapy such as docetaxel, 5-fluorouracil, paclitaxel-carboplatin, and cisplatin-gemcitabine have been completed. In general, these phase I trials have explored the combination of 250 and 500 mg of gefitinib in combination with full dose of the reference regimen. Treatment with gefitinib in combination with chemotherapy was well tolerated without any apparent increment in toxicity. Concurrent pharmacological studies failed to reveal any pharmacological interactions.

A phase II study assessed gefitinib monotherapy in patients with advanced SCCHN, documenting a response rate of 10.6% and a disease control rate of 53% [47]. Clinical trials in other tumour types have also been conducted, including glioblastoma [48], prostate [49], and colorectal cancer [50]. Although no objective responses have been documented in these trials, several patients had tumour markers decrements, suggesting antitumour activity. Numerous clinical trials evaluating combined therapy of gefitinib with radiation therapy and chemotherapy are underway. Two phase II studies have evaluated the clinical activity of gefitinib at two dose levels (250 and 500 mg) in

Table 2. Small Molecules Targeted to the Epidermal Growth Factor Receptor Tyrosine Kinase and HER-2

Drug	EGFR IC ₅₀ (µM)	HER-2 IC ₅₀ (µM)	Interaction
Gefitinib (Iressa TM , ZD1839)	0.02	3.7	Reversible
Erlotinib (Tarceva TM , OSI-774)	0.02	3.5	Reversible
PKI-166	0.02	-	Reversible
EKB-569	0.04	1.2	Irreversible
GW2016	0.01	0.009	Reversible
CI-1033	0.0008	0.02	Irreversible

patients with NSCLC that had failed at least one (210 patients) and at least two (216 patients) chemotherapy regimens for advanced disease, documenting response rates of 18.7% and 10.6%, respectively [51, 52]. In these studies, a higher dose did not improve response rate and caused an increased toxicity. Improvement in disease-related symptoms was significant in both trials. These results led to the regulatory approval of gefitinib 250 mg/d as monotherapy treatment for patients with locally advanced or metastatic NSCLC refractory to platinum-based and docetaxel chemotherapy. However, the addition of gefitinib to standard chemotherapy has failed to induce an improvement in response or survival in chemo-naïve NSCLC patients. Two placebo-controlled, double-blinded, phase III randomised trials evaluating chemotherapy (either gemcitabine-cisplatin or paclitaxel-cisplatin) plus either gefitinib (250-500 mg) or placebo have rendered negative results [53, 54]. Nevertheless, the clinical development of gefitinib in patients with NSCLC has continued, and trials evaluating sequential treatment with chemotherapy and gefitinib versus chemotherapy alone are underway. Recent data have shown that mutations in the ATP-binding site of the *egfr* gene predict sensitivity of NSCLC patients to gefitinib [55, 56]. In two retrospective analyses involving 16 and 66 patients with refractory NSCLC treated with gefitinib, 8/9 and 5/5 responders carried a mutation in the EGFR ATP-binding site, whereas 0/8 and 0/5 of the non-responders had such alterations, respectively. In the first report, cell lines were transfected with such mutations, and mutant strains showed equivalent sensitivity to gefitinib concentrations 10-fold lower than parental cell lines. Parental EGFR was inhibited by 50 percent at a gefitinib concentration of 0.1 μM and was completely inhibited by a concentration of 2.0 μM , whereas the IC_{50} values for the mutant EGFR were 0.015 μM and 0.2 μM , respectively. These results may indicate that susceptibility to the drug is only observed in those subjects carrying mutations that render the EGFR susceptible to clinically achievable drug concentrations, which are suboptimal to efficaciously inhibit the receptor in the vast majority of the patients without the described mutations.

Erlotinib (OSI-774; Genentech, San Francisco, CA) is a quinazoline derivative, which reversibly inhibits the kinase activity of EGFR. It has shown *in vitro* and *in vivo* activity in preclinical trials in multiple human cancer cell lines, including ovarian, head and neck, and non-small cell lung carcinoma [57, 58].

Erlotinib has been evaluated in several phase I studies using different doses and schedules, including weekly administration for 3 weeks every 4 weeks, and a continuous daily dosing [59]. The schedule that was ultimately chosen for further evaluation consists on the daily administration of 150 mg orally, with higher doses resulting in dose-limiting diarrhoea and cutaneous acneiform rash [60]. The cutaneous toxicity was dose-dependent, affected the face and upper trunk areas, appeared at the end of the first week of dosing and progressively recovered even in patients who continue taking the same dose of erlotinib. Other toxicities were mild to moderate and consisted of nausea and vomiting, elevation in bilirubin, headaches, and mucositis.

The preliminary results of several disease-directed studies have been presented. Erlotinib has demonstrated clinical

activity as single agent in patients with NSCLC, SCCHN, and ovarian cancer [61-63]. However, in these phase II studies there was a statistically significant association between the development of rash and the overall survival of patients. A combined analysis of the data showed that patients who develop rash of any grade had a statistically significant longer median survival [64]. As a consequence, intense research efforts are being undertaken to elucidate the pharmacodynamic basis of these findings, and to develop and implement pharmacodynamically-based endpoints for future trials.

Erlotinib is currently undergoing phase III evaluation in a great variety of solid malignancies and recently was classified as orphan drug for the treatment of glioblastoma. Interestingly, glioblastoma is at present the only tumour type in which amplification of the EGFR gene has been consistently documented, and it is associated with the production of a EGFR mutant protein (EGFRvIII) that has a truncated extracellular domain and constitutive tyrosine-kinase activation [65]. Erlotinib has demonstrated activity against this mutant form of EGFR. Preliminary data on two phase III clinical trials in patients with non-small cell lung cancer comparing standard chemotherapy regimens with or without erlotinib showed that this approach has failed to demonstrate a response or survival advantage [66]. On the other hand, preliminary data from a trial that randomised patients with refractory NSCLC to erlotinib or placebo in a 2:1 ratio has shown a significant increase in overall survival, response rate, and cancer-related symptoms in the erlotinib arm.

EKB-569 is an oral, selective and irreversible EGFR inhibitor. In an initial phase I study EKB-569 has been reported to be safe both on an intermittent and continuous-dose schedule. The agent is well tolerated with diarrhoea and acneiform rash being the most common reported toxicities. Grade 3 diarrhoea was the dose limiting toxicity at doses of 100 mg/day with 75 mg/day being the recommended dose for future studies [67]. In a phase I/II trial of EKB-569 in combination with irinotecan, 5-fluorouracil, and folinic acid (FOLFIRI) in patients with colorectal carcinoma, the DLTs were grade 3 diarrhoea and fatigue, and a modified FOLFIRI regimen and a daily dose of 25 mg was the recommended dose. Of 39 patients evaluated, a 38% objective response rate was documented. EKB-569 treatment resulted in complete inhibition of pEGFR and significant inhibition of pMAPK in both skin samples (11 pts) and tumour samples (3 pts) with no change in pAKT activity [68].

GW572016, also known as GW2016, is a 6-thiazolyquinazoline that reversibly inhibits the phosphorylation of both EGFR and HER2. It has demonstrated potent antitumour growth inhibitory activity both *in vitro* and *in vivo*. This agent is currently undergoing clinical evaluation, and in early clinical trials has shown a typical TKI toxicity profile, with diarrhoea and rash being the most relevant adverse events [69]. Disease-directed studies in breast and colorectal cancer patients are underway [70].

CI1033, also called PD183805, is a small molecule that irreversibly inhibits the three members of the HER family with tyrosine-kinase activity (HER1, 2 y 4) [71]. Phase I studies using different oral administration schedules have been conducted, showing a toxicity profile consisting in

mild to moderate vomiting, diarrhoea, vasculitis, and acneiform rash [72-74]. This compound is undergoing disease-directed evaluation in several tumour types, including breast cancer.

2.2. Targeting HER2

The HER2 (also known as c-erbB-2, or *neu*) gene has been shown to be amplified in human breast cancer cell lines. HER-2 is dramatically amplified (from 2- to greater than 20-fold) in 30% of breast cancer tumours [75]. Amplification of the HER-2 gene is an independent predictor of a shorter overall survival and time to relapse in patients with breast cancer. HER-2 is overexpressed in a significant proportion of patients (10% to 45%) in other malignancies, including NSCLC, ovarian, and gastric cancer. Also, HER-2 status predicts outcome in patients with ovarian [76] and gastric cancer [77].

Trastuzumab (Genentech, San Francisco, CA), is a MAb that targets the extracellular domain of HER2. After a series of phase II trials alone and in combination, trastuzumab was evaluated in a pivotal trial in a randomised fashion in conjunction with chemotherapy in patients with HER2-overexpressing tumours. Trastuzumab treatment increased the clinical benefit of first-line chemotherapy in terms of progression-free and overall survival [78]. Trastuzumab represents the first successful HER-targeted therapy, and the pivotal trials were the proof-of-principle for the integration of targeted therapies in the management of patients with cancer.

2C4 is a humanized anti-HER2 MAb that binds to a different HER2 epitope than trastuzumab [79]. 2C4 inhibits the heterodimerization of HER2 with other HER membrane receptors, including EGFR, a feature that could result in efficacy against tumours with low expression of HER2. Initial clinical studies have evidenced clinical activity in patients with solid tumours [80]. Phase II trials are underway in patients with a variety of solid tumour types.

3. PDGFR/BCR-ABL/C-KIT: STI571 (IMATINIB, GLEEVEC™)

STI571 was initially designed as an inhibitor of the platelet-derived growth factor receptor (PDGFR). However, it was evidenced that the compound was able to efficiently inhibit the tyrosine kinase activity of c-kit (CD117), *abl*-related tyrosine kinase activities (p210, p185, *v-abl*, *c-abl*), and the chronic myeloid leukaemia (CML) anomalous fusion protein *bcr-abl*. Imatinib presents a favourable oral bioavailability, with a half life of 13-16 hours. The toxicity profile includes nausea, myalgia, oedema, cutaneous rash, and, rarely, anemia and alterations in liver function tests. Episodes of intratumoural bleeding have been described, specially in the context of dramatic clinical responses.

In patients with CML imatinib is highly effective inducing haematological responses (up to 98%), although complete cytogenetic responses are less frequent. Also, imatinib shows a lower level of efficacy in patients with blast crisis (responses in 40-60%), especially in those with lymphoid phenotype [81]. A randomised, phase III

comparison of imatinib with interferon- α plus cytarabine as initial treatment for newly diagnosed chronic-phase CML, which demonstrated significantly higher rates of disease response with less toxicity, better quality of life, and a significantly longer progression-free survival time, provided the most persuasive data supporting a major role for imatinib [82]. Kantarjian *et al.* reviewed 261 patients with chronic phase CM) post interferon- α (IFN) failure treated with imatinib 400 mg daily. With a median follow-up time of 45 months, the major cytogenetic response rate was 73%, and the complete cytogenetic response rate 63%. Compared with a historical group of 251 similar patients treated with non-imatinib therapies, imatinib was associated with a better 4-year survival rate (86%, versus 43% $p < 0.0001$); the survival advantage was confirmed by multivariate analysis (hazard ratio 0.19, $p < 0.0001$) [83]. However, allogenic stem cell transplantation is the only treatment modality with long-term data demonstrating curative potential in CML.

The use of imatinib is less controversial in patients with gastrointestinal stromal tumours (GIST), a relatively uncommon malignancy characterized by unresponsiveness to chemotherapy, and a high dependence on a mutation in the c-kit receptor (CD-117) that induces a constitutive activation of its tyrosine kinase activity. An open-label, randomized, multicenter trial to evaluate the activity of imatinib in patients with advanced gastrointestinal stromal tumour was performed, and a total of 147 patients were randomly assigned to receive 400 mg or 600 mg of imatinib daily. Overall, 79 patients (53.7 percent) had a partial response, and an additional 41 patients (27.9 percent) had stable disease. The median duration of response had not been reached after a median follow-up of 24 weeks after the onset of response. Early resistance to imatinib was noted in 20 patients (13.6 percent). There were no significant differences in toxic effects or response between the two doses [84]. The results of this trial led to the accelerated approval of imatinib as first-line therapy for patients with advanced GIST. However, surgery remains the only curative therapeutic modality available, and a key strategy for prolonging the survival of patients with GIST may be to improve the outcome of surgery. Therefore, the role of adjuvant and neoadjuvant of imatinib in the overall management approach to advanced GIST is being investigated [85].

Resistance to imatinib can develop through several mechanisms, the most common of them being point mutation in the target receptors that either increase the expression or induce reactivation of the TK activity by increasing several fold the IC_{50} required for inhibition (in an inverse manner as point mutations in the EGFR TK influence response to gefitinib) [86, 87]. This latter mechanism may in part justify the approach of increasing the dose in patients that develop disease progression while on relatively low initial doses. Imatinib has also shown clinical efficacy in haematological malignancies with rearrangements of the PDGFR [88].

4. VEGF

Tumour growth depends on angiogenesis – the development of new vessels and cancer cells begin to promote this process early in tumorigenesis [89]. This

angiogenic impulse is characterized by oncogene-driven tumour expression of pro-angiogenic proteins, including vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), basic fibroblast growth factor (bFGF), transforming growth factor (TGF- β), platelet-derived growth factor (PDGF), placenta-like growth factor (PLGF), among others [90].

The human VEGF (*VEGFA*) gene is located on chromosome 6p21.3. *VEGFA* belongs to a family of genes that also include placental growth factors 1 and 2 (*PLGF1* and *PLGF2*), *VEGFB*, *VEGFC*, and *VEGFD*. Alternative splicing of the VEGF mRNA yields four different isoforms, VEGF121, VEGF165, VEGF189, and VEGF206 [91]. VEGF121 and VEGF165 are free to diffuse into the extracellular space. VEGF165 represents the major VEGF activity [92]. VEGF gene expression is regulated by several mechanisms. Under physiological conditions, VEGF is upregulated and its mRNA stabilized only after hypoxia [93]. In tumour tissues, by contrast, VEGF is constitutively overexpressed, independently of the environmental oxygen tension, but can be further increased by hypoxia [94]. Hypoxia-induced VEGF upregulation is mediated by HIF1, an event negatively regulated by the von Hippel Lindau (VHL) protein. The absence of VHL induces vascular abnormalities and renal cell cancer. VEGF mRNA expression is upregulated by a wide array of oncogenes (including H- and K-*ras*, *v-raf*, *src*, *PTEN*, *p53*, *Wnt*, and *c-jun*, among others), and growth factors (including EGF, TGF- β , TGF- α , IGF-1, and PDGF), suggesting the existence of auto- and paracrine loops. A high VEGF expression level is associated with a worse outcome in several malignancies, including colorectal cancer, NSCLC [95], and non-Hodgkin lymphoma [96]. VEGF is an attractive target for anti-angiogenic therapy because its receptors are present almost exclusively on genetically-stable, non-neoplastic endothelial cells, and are upregulated in tumour vessels when compared with normal endothelium [97]. This genetic stability may decrease acquired drug resistance incidence. A series of experiments demonstrated that treatment with anti-VEGF monoclonal antibodies reduced the growth of human tumours (glioblastoma and rhabdomyosarcoma) xenografted in mice, providing evidence that tumour growth is angiogenesis-dependent [98, 99].

VEGF binds two related receptors with tyrosine kinase activity, VEGFR-1 (flt1) and VEGFR-2 (KDR). VEGFR-3 (flt4) is a member of the same family, but does not bind VEGF, but VEGFC and VEGFD, and is involved in the regulation of lymphangiogenesis. VEGFR-1 expression is upregulated by hypoxia by the hypoxia-inducible factor (HIF1) [100]. An alternatively spliced, soluble form of VEGFR-1 is an inhibitor of VEGF activity. Some authors believe that VEGFR-1 functions primarily as a decoy receptor, regulating in a negative fashion the activity of VEGF on the endothelial cells, by preventing VEGF binding to VEGFR-2. Upon activation VEGFR-2 undergoes dimerization and ligand-dependent phosphorylation, subsequently inducing the phosphorylation-mediated activation of several intracellular pathways, including SRC, PI3K, and Raf/Mek/Erk. VEGFR-2 is considered to be the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF, and hence is a major target for antiangiogenic therapies. Also, the levels of

the VEGF receptor are correlated with a with a poorer grade of tumour differentiation and prognosis in pancreatic cancer [101].

A number of plasmatic surrogate markers of efficacy are currently under evaluation (such as VEGF, von Willebrand protein, or sVEGFR-1), as well as functional and morphological techniques (such as positron emission tomography [PET] scan or magnetic resonance imaging [MRI] techniques).

4.1. Targeting VEGF

Considering the critical role of VEGF in tumour development, and the recently reported positive impact of VEGF targeted therapy, this is one of the most exciting fields of cancer investigation nowadays. A number of strategies are being pursued to target VEGF, including monoclonal antibodies and soluble forms of the VEGF receptor.

Bevacizumab (rhMAB-VEGF; Genentech, San Francisco, CA) is a recombinant, humanized monoclonal antibody directed against VEGF. In phase I studies bevacizumab showed a low incidence of severe toxicities, with a markedly vascular profile (intratumoural bleeding, pulmonary emboli, and venous thrombosis). In a phase II study of bevacizumab in combination with gemcitabine in patients with pancreatic cancer a response rate of 38% was documented, along with a remarkable 54% 1-year survival rate [102]. A phase I/II trial evaluated bevacizumab monotherapy in patients with previously treated metastatic breast cancer, showing an overall response rate of 6.7%, and a median duration of response of 5.5 months [103]. Bevacizumab in combination with capecitabine showed a superior response rate compared with capecitabine alone (19.8% vs. 9.1%) in 486 patients with anthracycline- and taxane-refractory breast cancer [104]. Two Phase II trials of bevacizumab and erlotinib in patients with metastatic renal carcinoma (RCC) and NSCLC have evidenced remarkable response rates (25% and 18%, respectively), supporting the concept of combined inhibition of critical pathways [105, 106]. In a Phase II trial combining bevacizumab and gemcitabine in patients with pancreatic cancer a 21% response rate has been documented [107]. These promising results have prompted the implementation of a randomized phase III trial by the CALGB, comparing gemcitabine alone versus gemcitabine plus bevacizumab. Another phase II study of single agent bevacizumab compared with placebo in patients with metastatic renal carcinoma was prematurely closed in view of the results of an interim analysis that evidenced a significant improvement in progression-free survival, although no impact on overall survival was documented [108]. These results are particularly relevant considering that the rationale for choosing renal cancer as a target was that mutations in the tumour-suppressor gene VHL causing VEGF upregulation are highly prevalent both in hereditary and sporadic cases. The results of a phase III study that compared chemotherapy (irinotecan, 5-fluorouracil, and leucovorin, [IFL]) plus bevacizumab or placebo as first-line therapy in patients with metastatic colorectal carcinoma have been recently communicated [109]. The addition of bevacizumab to standard chemotherapy resulted in increased overall survival, progression-free survival, response rate and duration of response compared

with chemotherapy alone. Regarding toxicity, grade 3 hypertension and bowel perforation were significantly more prevalent among patients receiving the biological agent. This is the first antiangiogenic agent that induces an increase in overall survival.

VEGF-Trap is a fusion protein consisting of portions of the human VEGFR-1 and VEGFR-2 extracellular domains fused to the Fc portion of a human IgG1. It acts by binding and inactivating VEGF in the circulation and in tissues, and effectively suppresses tumour growth and vascularization *in vivo*, resulting in stunted and almost completely avascular tumours [110]. Preliminary data from a phase I study in patients with solid tumours and lymphoma show a favourable toxicity profile [111].

4.2. Targeting VEGFR

Another key strategy in the development of angiogenesis modulators is targeting the transduction of the signal triggered by VEGF. There are several classes of VEGFR targeting drugs under evaluation, including small molecules with selective TKI activity, and a selective ribozyme that cleaves the VEGFR-1 mRNA. Some have reached advanced phases of clinical development (Phases I and III), but others have shown disappointing results, like SU5416, a promising VEGFR TKI whose development was stopped due to an unexpectedly high incidence of severe toxicity in a phase III trial in combination with chemotherapy.

SU11248 is an oral multi-targeted TKI with anti tumour and anti angiogenic activity through targeting PDGFR, VEGFR, KIT and FLT3. SU11248 has been evaluated in a phase I study where 28 patients with solid tumours were treated, observing 6 radiological responses (renal cell carcinoma and neuroendocrine tumours) [112]. In a phase I/II study in 32 patients with gastrointestinal stromal tumours refractory to imatinib, 11 responses or long-lasting stabilization of disease were documented [113]. Although the multimodality of action of this drug obscures each pathway's contribution to the achievement of a biological effect, VEGF plasma levels consistently rose after therapy in both studies, possibly indicating the triggering effect of hypoxia on VEGF synthesis.

SU6668 is a small molecule inhibitor of the angiogenic receptor tyrosine kinases VEGFR-1, PDGFR- α , and FGFR-1 [114]. Up to four different phase I trials have been reported in abstract form, evidencing a mild toxicity profile, and phase II evaluation is underway.

PTK787/ZK222584 is an oral angiogenesis inhibitor that selectively targets VEGFR-1, VEGFR-2, and VEGFR-3 tyrosine kinases. The results of a disease-directed phase I trial in 43 patients with glioblastoma multiforme have been communicated, showing 1 partial response and 20 disease stabilizations. Most importantly, dynamic MRI scanning showed decreases in vascular permeability and cerebral blood volume post-treatment, and decreases in both parameters at day 30 appeared dose-dependent [115]. In a recently published phase I study the pharmacodynamic effects of PTK787/ZK222584 were evaluated by assessing contrast-enhancement parameters of metastatic liver lesions using dynamic MRI in patients with advanced colorectal cancer

treated in two ongoing, dose-escalating phase I studies, finding that patients with a best response of stable disease had a significantly greater reduction in tumour permeability and vascularity parameters compared with progressors [116].

CEP-7055 is the dimethylglycine ester of CEP-5214, a TKI with nanomolar potency against VEGFR-1, 2 and 3 [117]. Preliminary results from a phase I study assessing a twice daily schedule have evidenced a favourable toxicity profile [118].

ZD6474 is an orally available inhibitor of the tyrosine kinase activity of both the VEGFR-2 and the EGFR [119]. Preliminary observations of tumour regression have been documented in 4 patients with NSCLC out of 18 enrolled in a phase I study [120].

Angiozyme is a ribozyme that downregulates the VEGFR-1 function by specifically cleaving the pre-mRNAs for the primary VEGFR-1 and reduces both the cell surface receptor and its truncated, soluble form (sVEGFR-1). It has been evaluated in 83 chemonaïve colorectal cancer patients in combination with IFL, documenting a 43% response rate [121]. Interestingly, patients that presented detectable levels of sVEGFR-1 at baseline that converted to undetectable after therapy fared significantly better.

5. SUMMARY

An enormous body of knowledge has been gathered in the past 20 years that has enabled us to successfully develop rationally designed therapies for cancer patients, and the results of their clinical evaluation are now becoming available. The lack of positive results of some of these trials that have evaluated the introduction of targeted therapies in conjunction with chemotherapy have prompted the implementation of novel trial designs. These include early discontinuation studies, where patients that show stable disease after targeted therapy are randomized to continue or discontinue treatment, and sequential trials, where patients whose tumours respond or are stabilized with standard chemotherapy are randomized to maintenance targeted therapy or placebo. The success of the trastuzumab and bevacizumab studies has proven that selection of patients is critical in order to demonstrate the potential advantages of a given drug. A poor selection strategy may dilute the clinical effect of a potentially effective therapy. Research efforts have to focus not only in the pursue of new therapeutic alternatives, but also in the development of reproducible tools that allow us to prospectively identify the subset of patients that are more prone to obtain a benefit from a given intervention.

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