Combining molecular targeted therapies: clinical experience

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Approximately 20 molecular targeted therapies, mainly monoclonal antibodies and tyrosine kinase inhibitors, have been approved for the treatment of various cancers. They are being increasingly investigated in combination in clinical trials. We review the rationale for combining molecular targeted therapies and the results of clinical trials to date. There have been some exciting clinical results with some combinations, for example, lapatinib/trastuzumab or bevacizumab/trastuzumab in HER2-positive metastatic breast cancer, whereas other potential combinations have provided disappointment, for example, cetuximab or panitumumab in combination with bevacizumab/chemotherapy in first-line treatment of metastatic/advanced colorectal cancer. Therefore, at this point no general guidance to study such combinations can be derived. ***Anti-Cancer Drugs*** **22:701–710 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Approximately 20 molecular targeted therapies (MTTs) have been approved for medical use in the treatment of cancers over the last 15 years (Table 1) [1]. The clinical experience acquired with these agents has established their specificity. The efficacy of tyrosine kinase inhibitors (TKIs) is best observed when the target is constitutively activated by a mutation or a translocation and is a major driver for transformation and tumor progression. For antigrowth factor monoclonal antibodies, the relationship between efficacy and overexpression of the target is often established (i.e. trastuzumab and HER2), although not ascertained when ubiquitary targets are involved (i.e. cetuximab and receptor epidermal growth factor). MTT-induced adverse effects, some of which are subacute and not predicted from preclinical investigations and toxicological studies, show lack of absolute specificity by MTTs for cancer tissues. Common adverse effects across all MTT classes are skin toxicity, diarrhea, fatigue, and transaminitis, whereas hypertension, thromboembolic events, proteinuria, and hemorrhagic episodes are a hallmark of the toxicity with antiangiogenic therapies [2].

The increasing use of MTTs in clinical practice has pointed to a number of limitations to achieve efficacy. The molecular target must be in a configuration that is accessible to the agent, such as the activating mutations of epidermal growth factor receptor (EGFR) in non-small cell lung-cancer (NSCLC) or of c-KIT in gastrointestinal stromal tumors [3,4]. Furthermore, no downstream activation of the transduction pathway should exist [5,6]. It is important that circulating drug concentrations are sufficiently high to durably inhibit the target in cancer tissues [7,8]. In advanced cancers, long-term exposure to a single MTT can be ultimately limited by the development of resistance through numerous different mechanisms. Resistance can be related to a decreased expression of the target [9] or the development/selection of tumor cell populations harboring mutations of the gene associated with MTT resistance [10–13]. It can be related to the selection of a tumor cell population with activation of a transduction pathway bypassing the targeted one [14], pointing to a theoretical interest for inhibition of more than a single target at the same time either by combining specific MTTs or by using multitargeted inhibitors.

However, the rationale for such combinations is not straightforward. With multikinase inhibitors, it would seem that inhibition of a single kinase accounts for the largest part of, if not all, the antitumor activity in a given tumor, in addition, the key kinase related to the activity can be variable according to the targeted cancer. For instance, the multikinase, imatinib, performs its activity based on BCR-ABL in chronic myelogenous leukemia, c-KIT in gastrointestinal stromal tumor, and platelet-derived growth factor receptor in dermatofibrosarcoma protuberans [15]. Furthermore, multikinase inhibitors are often more toxic as they produce more off-target effects, the greater specificity of single kinase inhibitors results in fewer off-target effects and lesser toxicity. Nevertheless, it is rational to suggest a combination of separate single kinase inhibitors. By this approach, it seems easier to optimize relevant blockades, to achieve maximum blockade of one transduction pathway through inhibition of sequential transduction steps, to simultaneously target a transduction pathway and its escape mechanisms, to

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Table 1 Approved molecular targeted therapies

Target	INN	Entity	Indications
HER receptor family			
Anti-EGFR	Cetuximab	Humanized MoAB	wt-KRAS CRC
			Head and neck cancer
Anti-EGFR	Panitumumab	Human MoAB	wt-KRAS CRC
Anti-HER2	Trastuzumab	Humanized MoAB	HER2-positive breast cancer (adjuvant, metastatic)
EGFR TKI	Erlotinib	NCE	Second-line NSCLC
			Pancreatic cancer
EGFR TKI	Gefitinib	NCE	NSCLC with activator EGFR mutation
HER/EGFR TKI	Lapatinib	NCE	HER2-positive breast cancer after failure of trastuzumab
Multikinase TKI			
Multikinase TKI, predominantly antineoplastic			
PGDFR, ABL, c-KIT	Imatinib mesylate	NCE	Ph+ CML/ALL
			MDS/MPD
			Hypereosinophilic syndrome with PDGFR rearrangement
			GIST with activating c-Kit mutation
			Unresectable DFSP
PGDFR, ABL, c-KIT, SRC, EPHRIN	Dasatinib	NCE	Ph+ CML
			ALL resistant or intolerant to earlier therapy
PGDFR, ABL, c-KIT, EPHRIN	Nilotinib	NCE	Ph+ CML resistant or intolerant to earlier therapy
Multikinase TKI, predominantly antiangiogenic			
PDGFR, VEGFR, CSF, RET	Sunitinib	NCE	RCC
			GIST resistant or intolerant to earlier therapy
CRAF-BRAF, c-KIT, FLT-3, VEGFR-2	Sorafenib	NCE	Hepatocellular cancer
			Second-line RCC
KIT, VEGFR, PDGFR, wt-RAF	Pazopanib	NCE	RCC
Anti-VEGF MoAB			
	Bevacizumab		Metastatic CRC
			NSCLC
			First metastatic progression of breast cancer
			RCC (combined with interferon α)
Proteasome inhibitor			
	Bortezomib	NCE	Myeloma
Anti-CD20			
	Rituximab	Humanized MoAB	B-NHL
			CLL
	Ofatumumab	Human MoAB	Relapsed CLL
MTOR inhibitors			
	Everolimus	NCE	Second-line RCC
	Temserolimus	NCE	RCC

ALL, acute lymphoblastic leukemia; BNHL, B-cell nonHodgkin lymphoma; CLL, chronic lymphocytic leukemia; CML chronic myelogenous leukemia; CRC, colorectal cancer; DFSP, dermatofibrosarcoma protuberans; EGFR, epithelial growth factor receptor; GIST, gastrointestinal stromal tumor; HER, human epidermal growth factor receptor; INN, International nonproprietary name; MDS, myelodysplastic disease; MoAB, monoclonal antibody; MPD, myeloproliferative disease; MTOR: mammalian target of rapamycin; NCE, new chemical entity; NSCLC, non-small cell lung cancer; PGDFR, platelet-derived growth factor receptor; Ph+, Philadelphia chromosome-positive; RCC, renal cell cancer; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; wt-KRAS, wild-type *KRAS* gene.

target different transduction pathways that may be significant in a given tumor type, to impact extracellular targets with a monoclonal antibody and intracellular inhibition simultaneously, and to target other signals in a potentially additive manner [16].

Furthermore, with respect to antigrowth factor monoclonal antibodies, it should be realized that several mechanisms of action can be involved in their activity, for example, antibody-dependant cellular cytotoxicity, inhibition of activation through dimerization, competition with the natural ligand(s), inhibition of extracellular domain cleavage, and modulation of endocytosis and degradation. Various monoclonal antibodies directed against the same target are therefore not necessarily equal in their effect, thus providing a hypothetical basis for their combination [17].

There are a number of difficulties that arise when examining the combination of different agents. There are few preclinical models that simultaneously harbor the possible targets; the quantitative expression and/or the activation status of each target in a model may not be relevant to that observed in humans; and there are no preclinical models that reproduce the heterogeneity of human tumors. There is a poor degree of understanding of preclinical and clinical pharmacodynamic assessment of the activity of agents in relation to actual efficacy as it relates to tumor shrinkage or growth inhibition. It is currently impossible to predict new toxicities or possible additive toxicity. Potential pharmacokinetic interactions between agents can be expected and need to be investigated, especially with the large number of TKIs being metabolized by cytochrome P450 3A4 [18].

There are a number of prerequisites that need to be considered before advancing to the clinical investigation of a particular combination of agents. The following are some of the main prerequisites that need to be considered: there should be evidence for preclinical or clinical activity with the individual agents against human

tumors; pre-clinical models should provide evidence of additivity or synergism between the agents or an ability to overcome the development of acquired resistance; no deleterious pharmacodynamism or pharmacokinetic interactions should emerge; and moreover, no preclinical reason to suspect any unexpected toxicity should exist.

Combined multiple inhibition of HER transduction pathways

The erythroblastic leukemia viral oncogene homolog family of receptor tyrosine kinases encompasses four receptors, namely HER1 (EGFR), HER2, HER3, and HER4, which have been implicated in a variety of cancers. HER2 has no known ligand and HER3 has no tyrosine kinase activity. All share considerable homology in their kinase domains but not in their extracellular and C-terminal domain, thus allowing variable targeting by specific monoclonal antibodies and by specific and nonspecific TKIs.

Humanized or human monoclonal antibodies against EGFR (cetuximab, panitumumab) or HER2 (trastuzumab, pertuzumab being still in preregistration clinical trials) and several TKIs directed against EGFR tyrosine kinase (gefitinib, erlotinib) or against EGFR and HER2 tyrosine kinase (lapatinib) have been approved for clinical use or are about to be approved. Other dual or pan-HER TKIs such as neratinib are still undergoing investigation in clinical trials [19]. The existing target is not sufficient to warrant the efficacy of such therapies. Several biological mechanisms related to the target are involved in the sensitivity or the resistance. For instance, it is now well established that sensitivity mechanisms to EGFR TKIs such as activating mutations are present in NSCLC for the activity to be observed, and that resistance mechanisms such as loss of the extracellular domain, inhibiting mutations, or downstream activation of transduction pathways (activating KRAS mutations in colorectal cancer, activation of phosphatidylinositol 3-kinase C (PI3KC), and de-novo mutations of EGFR) also exist [20]. It seems that the effect of inhibitors on the Ras/Raf/MAPK and PI3K/Akt pathways, the main transduction pathways, differs between monoclonal antibodies and/or small molecule TKIs, providing a rationale for a 'total blockade' using a combination of inhibitors [21]. Indeed, blocking one HER receptor with a monoclonal antibody and enhancing apoptotic cell death with a TKI should be more effective than either strategy alone [22] and should be able to overcome the development of acquired resistance [20,23,24].

As mentioned earlier, this inhibition can also be achieved by multikinase inhibitors or dual inhibitors such as lapatinib, neratinib, or pan-HER inhibitors [25–27]. Dual HER1/HER2 targeting has been validated preclinically in HER2-positive breast cancer cell lines, prostate cancer cell lines, and some colorectal cancer cell lines. However, the activity observed so far with lapatinib seems to be mostly dependent on HER2 inhibition and minimally, if at all, upon EGFR inhibition [28]. Alternatively, most of its adverse effects, and particularly, skin and gastrointestinal toxicities seem to be related to EGFR inhibition [29].

Dual HER1/EGFR inhibition

Dual EGFR inhibition has been shown to be feasible in phase 1 studies with a combination of cetuximab and erlotinib in patients with advanced solid malignancies or cetuximab and gefitinib in patients with refractory NSCLC [30,31]. Conventional doses of each agent (250 mg/m^2 cetuximab intravenously weekly; 150 mg erlotinib orally daily; 250 mg gefitinib orally daily) were achieved but cutaneous toxicity, generally grade 1/2, seemed to be more frequent. No pharmacokinetic interactions were noted [30]. Another phase 1 study with the triple combination of cetuximab, erlotinib, and bevacizumab in patients with advanced solid tumors found that the maximum tolerated dose of erlotinib was reduced (50 mg daily) in combination with standard doses of cetuximab (250 mg/kg^2 intravenously weekly) and bevacizumab (IV 10 mg/kg every 2 weeks) [32]. To date, however, there are no published phase 2/3 data available to show additivity of antitumor effects of combination of EGFR inhibition in various types of cancer, although such clinical trials are being conducted.

Dual HER2 inhibition

De-novo or acquired resistance to trastuzumab is a concern in HER2-positive cancer treated with trastuzumab. Several resistance mechanisms have been hypothesized or established: truncation of the HER2 extracellular domain, overactivation of alternative signaling routes (EGFR, HER3, insulin-like growth factor-1 receptor), increased downstream PI3K/AKT signaling, phosphatase and TENsin homolog inactivation, and alterations and mutations in the HER2 extracellular domain [17]. In these cases, the activity of lapatinib (and other HER2-directed TKIs) seems to be minimally affected and they might synergize with trastuzumab to prevent or overcome resistance [33]. The reduced cardiotoxicity of lapatinib compared with trastuzumab and limited additive cardiotoxicity when these agents are combined is of further interest [34].

Dual HER2 inhibition with the combination of trastuzumab and lapatinib was initially studied in women with HER2-positive trastuzumab-resistant breast cancer [35]. Daily doses of oral lapatinib (750–1500 mg/m^2) were tolerated with the usual side-effects (skin rash, diarrhea, and fatigue). In a subsequent phase 3 trial [36], 296 patients with HER2-positive metastatic breast cancer progressing on earlier trastuzumab-containing regimens were randomized to 1500 mg of lapatinib once daily or 1000 mg of lapatinib once daily in combination with trastuzumab, thus mimicking continuation of trastuzumab and addition of another agent when metastatic patients progress despite trastuzumab [37]. Median overall survival (OS) after treatment with lapatinib and

trastuzumab was 51.6 weeks compared with 39 weeks for lapatinib (hazard ratio: 0.75; 95% confidence interval: 0.53, 1.07; $P = 0.106$). It should be noted that crossover to the combination was permitted if progression occurred in the first 4 weeks of single-agent therapy, potentially underestimating the survival benefit of the combination. The same dose of lapatinib (1000 mg once daily) was not tolerated because of excessive grade 3 diarrhea in an adjuvant study of lapatinib and trastuzumab and weekly paclitaxel after dose-dense doxorubicin-cyclophosphamide in patients with HER2-positive breast cancer, [38] and it was recommended that the dose of lapatinib should be decreased to 750 mg once daily in continuing studies.

Initial experience with combination of trastuzumab and lapatinib did not show additive toxicity, particularly with respect to gastrointestinal and skin toxicities, and notably cardiotoxicity. In terms of efficacy, additivity was strongly suggested. This has prompted several clinical trials to validate this combination (Table 2) with the first results expected in 2010. So far, no safety concern has emerged. Interim data from the CHER LOB neoadjuvant trial have suggested no particular safety concerns with the trastuzumab/lapatinib combination, whereas the pathological complete response rate of 37% so far reported is not superior to the 67% reported with concurrent neoadjuvant combination of chemotherapy and trastuzumab [39].

Dual HER1/HER2 inhibition

Phase 1/2 studies have reported the combination of trastuzumab with escalating doses of erlotinib or gefitinib in patients with HER2-positive breast cancer [40,41]. No pharmacokinetic interactions were noted. Erlotinib or gefitinib could be administered with trastuzumab at the usual doses without increased toxicities during phase 1 investigation, although an increased incidence of skin rash with trastuzumab/erlotinib was subsequently noted. Therefore, the recommended starting dose of erlotinib was amended to 100 mg/day with a possibility of increasing to 150 mg/day after 3 weeks. There was no indication of additional clinical benefit with these combinations compared with trastuzumab alone.

There are few data available on dual HER1/HER2 inhibitors (lapatinib or neratinib) or pan-HER inhibitors (CI-1033). Phase 2 trials of lapatinib in patients with hepatocellular carcinoma [42] or HER1 and/or HER2-expressing metastatic salivary gland tumors [43], and CI-1033 in NSCLC [44] and ovarian carcinoma [45] showed lower than expected clinical activity. Thus, multitargeted HER signaling inhibition is irregularly additive in terms of activity despite preclinical evidence of such activity. Pan-HER inhibitors such as CI-1033 do not seem to show a broader spectrum of activity than specific inhibitors.

The combination of the multi-HER TKI neratinib and trastuzumab has been studied in a phase 1/2 trial in patients with HER2-positive advanced breast cancer that

Table 2 Clinical trials studying the combination of cytotoxic chemotherapy and of trastuzumab and lapatinib either alone or combined simultaneously or sequentially

Trial	Studied options	Trial phase	Disease stage	Completion
EGF103892	Escalating doses of lapatinib (750–1500 mg/day) in combination with paclitaxel and carboplatin with or without weekly trastuzumab	I	IV	December 2008
EGF100161	Escalating doses of lapatinib (750–1500 mg/day) in combination with docetaxel (75 mg/m^2 q3w) and weekly trastuzumab	I	IV	April 2010
EORTC10054	Arm I: FEC/docetaxel and lapatinib Arm 2: FEC/docetaxel and trastuzumab Arm 3: FEC/docetaxel and trastuzumab/lapatinib	I/II	III, neoadjuvant	December 2009
NCT00470704	Lapatinib and trastuzumab q3w	II	IV	May 2010
CHER LOB	Weekly paclitaxel × 12 then FEC plus trastuzumab and/or lapatinib	II, randomized	I–III, neoadjuvant	Completed
EGF104900	Lapatinib 1500 mg once daily vs weekly trastuzumab+lapatinib 1000 mg once daily	III	IV	Completed
NCCTG-N083E	Docetaxel, carboplatin, trastuzumab, lapatinib	II	I–II, adjuvant	April 2009
MCCRC-RC0639	AC followed by trastuzumab, paclitaxel, lapatinib	II	I–II, adjuvant	June 2009
MSKCC-07013	AC followed by trastuzumab, paclitaxel, lapatinib	II	I–III, neoadjuvant	March 2010
LPT109096	Trastuzumab and/or lapatinib. Sequential FEC75 and paclitaxel	II, randomized	I–III, neoadjuvant	
ALTTO	Lapatinib and/or trastuzumab in sequence or combination after 3 or more cycles of chemotherapy	III	I–II, adjuvant	June 2010
NeoALTTO	Paclitaxel and lapatinib and/or trastuzumab	III	I–III, neoadjuvant	September 2019
ONCOMO-0105	Any chemotherapy+lapatinib and/or trastuzumab	II, randomized	I–III, neoadjuvant	June 2009
NSABP-B41	AC followed by paclitaxel with trastuzumab and/or lapatinib	III	I–III, neoadjuvant	July 2014
EGF104383	Paclitaxel+trastuzumab and/or lapatinib	III	IV	September 2014
CALGB 40601	Paclitaxel+trastuzumab and/or lapatinib	III	I–III, neoadjuvant	September 2019

AC, doxorubicin–cyclophosphamide; FEC, fluorouracil–epirubicin–cyclophosphamide.

progressed after trastuzumab therapy. Neratinib (240 mg) taken orally daily could be combined with weekly trastuzumab without unexpected toxicities or left ventricular ejection fraction (LVEF) decrease. Among the 33 patients evaluable for efficacy, the objective response rates (ORR) was 27%, which is not significantly different from the ORR of 24% reported with neratinib alone in the same setting [46].

Combined sequential inhibition of HER transduction pathways

The possibility of sequential inhibition of HER receptor(s) and subsequent downstream signaling using PI3K or mammalian target of rapamycin inhibitors is a potentially attractive approach for circumventing or preventing HER inhibitor resistance. Despite preclinical investigations on combined HER and PI3K inhibitor, there are no clinical studies reported of these possible combinations. The combination of HER inhibitors and mammalian target of rapamycin inhibitors has shown additivity and reversal of resistance to HER1 inhibitors in preclinical models [47–49], but there have been few clinical studies. In a phase *2* trial [50], the combination of oral erlotinib (150 mg daily) and sirolimus (5–10 mg daily) in patients with recurrent glioblastoma was tolerated, the most common grade greater than or equal to *2* adverse events being skin rash (59%), mucositis (34%), and diarrhea (31%) with few grade 3 events, although there was negligible evidence of clinical activity. Phase 1 and *2* clinical trials have not reported any unexpected toxicity with the combination of gefitinib and everolimus in patients with NSCLC, and although responses were reported in the latter study, there have been no subsequent reports on the outcome [48].

Combined inhibition of HER and angiogenesis

Biological agents that target these individual pathways have proven effective in treating patients with NSCLC, colorectal cancer, and breast cancer. There is a crosstalk between EGFR and vascular endothelial growth factor (VEGF) pathways, and both can be activated in the same tumor [51]. Additivity has been observed in preclinical models [51,52]. The combination of EGFR and VEGF inhibition is therefore currently under investigation as a means to overcome resistance and promote synergy. Dual inhibition of EGFR and VEGF pathways can also be accomplished with a single agent, such as vandetanib or XL647, which targets both the pathways [53]. It has also been studied with the combination of a specific HER inhibitor (e.g. cetuximab, panitumumab, gefitinib, erlotinib, lapatinib) with an angiogenesis inhibitor, most frequently bevacizumab.

Combination of anti-EGFR and anti-VEGF monoclonal antibodies

Anti-EGFR monoclonal antibodies (cetuximab and panitumumab) have established activity in patients with advanced colorectal cancer, not harboring KRAS activating mutation [54]. In a phase *2* trial (the BOND-*2* study) [55], patients with irinotecan-resistant advanced colorectal cancer received cetuximab/bevacizumab in combination and randomization with or without irinotecan. The ORR observed was 20 and 37%, and the median time to tumor progression was 4.9 months and 7.3 months, respectively. This favorable clinical activity and the fact that no unexpected toxicity issues were revealed, stimulated interest and further investigation of such combinations.

However, results from two large-scale, prospective, randomized, phase 3 trials on addition of an anti-EGFR monoclonal antibody to first-line chemotherapy and bevacizumab in patients with advanced colorectal cancer yielded somewhat disappointing results. In the PACCE trial [56], patients ($n = 1053$) received irinotecan-based or oxaliplatin-based chemotherapy and bevacizumab and were then randomized to therapy with or without panitumumab. In the CAIRO*2* trial [57], patients ($n = 755$) received capecitabine/oxaliplatin and bevacizumab with randomization with or without cetuximab. Efficacy was inferior in the experimental arms receiving anti-EGFR monoclonal antibody in both the trials (Table 3); particularly, the median progression-free survival (PFS) (the primary endpoint) was significantly shorter regardless of the KRAS status. Furthermore, toxicity (particularly cutaneous reactions and diarrhea) and/or quality of life were negatively impacted by anti-EGFR monoclonal antibody therapy. Such combination therapies are therefore not recommended for the treatment of advanced colorectal cancer in clinical practice.

Combination of trastuzumab and bevacizumab in HER2-positive breast cancer

Subsequent to a phase 1 study which showed that bevacizumab (10 mg/kg every 2 weeks) could be combined with trastuzumab (*2* mg/kg weekly), a phase *2* trial tested this combination in the first-line treatment of 50 patients with HER2-positive advanced breast cancer [58]. The ORR was 48% and median time to tumor progression was 9.2 months. The most frequent adverse events of any grade were hypertension ($n = 30$), fever/chills/infusion reactions ($n = 18$), headache ($n = 17$), epistaxis ($n = 17$), fatigue ($n = 15$), and proteinuria ($n = 12$). Eighteen patients developed grade 3/4 hypertension and an equal number developed grade 1/2 cardiac adverse events, all of which were asymptomatic, whereas one patient developed a grade 4 cardiac adverse event. Thus, promising activity was observed with the bevacizumab/trastuzumab combination in the absence of cytotoxic chemotherapy, but with a safety profile less favorable than that observed with either of the two agents administered alone.

The combination of bevacizumab and trastuzumab is being intensively investigated in additional clinical trails, the results of which are awaited with interest. At least 20 studies have been registered (*clinicaltrials.gov*) examining

Table 3 Main efficacy results from randomized phase 3 trials of chemotherapy plus bevacizumab with or without anti-EGFR monoclonal antibody for the first-line treatment of metastatic colorectal cancer

	PACCE			CAIRO2		
Efficacy	Bevacizumab Oxaliplatin ($n=415$)	Bevacizumab Oxaliplatin Panitumumab ($n=410$)	Bevacizumab Irinotecan ($n=115$)	Bevacizumab Irinotecan Panitumumab ($n=115$)	Bevacizumab Capecitabine Oxaliplatin ($n=378$)	Bevacizumab Capecitabine Oxaliplatin Cetuximab ($n=377$)
Median PFS (months)	10.5	8.8	11.7	10.5	10.7	9.4
Median OS (months)	24.5	19.4	20.5	20.7	20.3	19.4
RR (%)	48	46	43	40	50	53

OS, overall survival; PFS, progression-free survival; RR, response rate.

bevacizumab/trastuzumab with cytotoxic chemotherapy and hormonal therapy in patients with HER2-positive breast cancer in the neoadjuvant, adjuvant, and metastatic settings.

Combination of trastuzumab and pertuzumab in HER2-positive breast cancer

Pertuzumab is an investigational humanized anti-HER2 monoclonal antibody undergoing clinical development, which targets a different extracellular HER2 domain to that of trastuzumab. Pertuzumab, unlike trastuzumab, inhibits tumor growth in the absence of HER2 overexpression by preventing ligand-stimulated formation of HER2 heterodimer. Pertuzumab showed limited activity in a phase 2 trial in patients with HER2-negative metastatic breast cancer [59]. However, a combination of pertuzumab (6 mg/kg every 3 weeks) and trastuzumab (2 mg/kg every week or 6 mg/kg every 3 weeks) showed activity in a phase 2, open-label study in 66 patients with HER2-positive metastatic breast cancer, whose disease had progressed during earlier trastuzumab-based therapy [60]. The ORR was 24% and median PFS was 5.5 months. Adverse events were invariably mild or moderate and the cardiac dysfunction was minimal, with no discontinuations due to cardiac-related adverse events. A phase 3 trial of trastuzumab and docetaxel with or without pertuzumab in first-line metastatic HER2-positive breast cancer is currently under way as it may provide enhanced antitumor activity in less pretreated patients and delay acquired resistance. A second phase 3 study assesses the addition of pertuzumab to the association of capecitabine and trastuzumab in patients who have a recurrence subsequent to an earlier exposure to taxaneand trastuzumab regimens.

Combination of EGFR TKIs and anti-VEGF monoclonal antibodies

The combination of erlotinib and bevacizumab has been studied in phase 1/2 studies in patients with a wide range of cancers including NSCLC, head and neck cancer, hepatocellular cancer, ovarian cancer, pancreatic cancer, renal cell cancer, breast cancer, and cancer of unknown origin [61–71]. In these trials, the recommended dose was used for both the agents; no additivity was expected in terms of toxicity from each agent, although the study in ovarian cancer had to be stopped because of bowel perforations. Promising response and stabilization rates were reported in NSCLC, head and neck cancer, and hepatocellular cancer.

However, the activity of bevacizumab/erlotinib combination has not been confirmed in randomized studies. In a phase 3 trial in 607 patients with pancreatic cancer [71], the addition of bevacizumab to the combination of gemcitabine and erlotinib modestly but significantly ($P = 0.0001$) increased median PFS by 1 month (4.6 vs. 3.6 months) but without any significant increase in median OS. No unexpected adverse events were observed from adding bevacizumab to gemcitabine/erlotinib. In a randomized phase 2 study of 120 patients with recurrent or refractory NSCLC [65], bevacizumab/erlotinib combination did not confer any significant PFS or OS benefit over bevacizumab/chemotherapy; interest in this combination could not therefore be confirmed in NSCLC [66].

A combination of bevacizumab/erlotinib and FOLFOX chemotherapy was attempted in a phase 2 study of 35 patients with metastatic colorectal cancer, but interpretation of efficacy was confounded by higher than expected withdrawal because of toxicity [67].

The triple combination of cetuximab/erlotinib/bevacizumab has been investigated in a phase 1 study of patients with advanced solid tumors, but toxicity did not allow the administration of usual erlotinib doses [31]. The triple combination of imatinib (400 mg daily), erlotinib (150 mg daily), and bevacizumab (10 mg/kg every 2 weeks) was found to be a tolerable therapy in a phase 1/2 trial of patients with advanced renal cell cancer, and there were early indications of activity.

In a phase 2 study of lapatinib (1500 mg daily) and bevacizumab (10 mg/kg every 2 weeks) in patients with HER2-positive metastatic breast cancer, the combination proved well tolerated and early efficacy results indicated promising activity.

Combination of anti-HER monoclonal antibodies and angiogenic receptor TKIs

Preclinical investigation has indicated additive or synergistic antitumor activity between the anti-EGFR (HER1)

monoclonal antibody, cetuximab, and the multitargeted TKI, sunitinib, in combination with irradiation in an orthotopic head-and-neck cancer model. However, there are few published clinical data on this potential combination. The combination of cetuximab (500 mg/m^2 every 2 weeks), sunitinib (25 mg daily), and irinotecan has been studied as a third-line treatment in 27 patients with metastatic colorectal cancer; no responses were observed, although increased diarrhea and fatigue were reported.

Combination of anti-HER and antiangiogenic receptor TKIs

Phase 1 clinical trials have reported the combination of sorafenib with gefitinib in patients with refractory NSCLC, and sorafenib with erlotinib in patients with recurrent glioblastoma multiforme. These studies showed that gefitinib or erlotinib had no effect on the pharmacokinetics of sorafenib. However, sorafenib reduced the maximum plasma concentration and area under the plasma concentration/time curve for erlotinib and gefitinib [72]. The mechanism and clinical relevance of this interaction is unknown, as the expected accumulation of maximum plasma concentration and area under the plasma concentration/time curve for erlotinib at steady state was not observed. The recommended dose was 400 mg of sorafenib twice daily with 250 mg of gefinitib once daily, which was well tolerated with promising efficacy [72]. However, in a study combining 400 mg of sorafenib twice daily with 150 mg of erlotinib once daily in patients with earlier untreated advanced NSCLC, grade 3/4 adverse events included not only fatigue, hand–foot reaction, and rash in 16% of the patients but also diarrhea and hypophosphatemia in 42% of the patients; an encouraging 24% response rate and 50% stabilization was observed, although interpretation is difficult in the absence of data on EGF_R mutations.

The combination of sunitinib and gefitinib has been evaluated in a phase 1/2 study in 42 patients with metastatic renal cell cancer [73]. Pharmacokinetic analyses did not indicate any drug interaction. The maximum tolerated doses of gefitinib was found to be 250 mg daily and of sunitinib was 37.5 mg daily. Dose-limiting toxicity with LVEF declined and fatigue occurred with the full dose of sunitinib (40 mg daily). The most common grade 3/4 adverse event was diarrhea (14% of patients). ORR was 37% and median PFS was 11 months, which seemed comparable with the efficacy expected with single-agent sunitinib in this setting.

There are several reports studying the combination of lapatinib and pazopanib. In a phase 1 study in patients with solid tumors, coadministration did not seem to affect the pharmacokinetics of lapatinib, although the bioavailability of pazopanib may be increased. In a phase 1/2 study in patients with recurrent malignant glioma, a preliminary optimal tolerated regimen was determined to be a combination of pazopanib (600 mg twice daily) and lapatinib (1000 mg twice daily). Larger scale randomized phase 2 studies of the pazopanib/lapatinib combination have been reported in patients with advanced and recurrent cervical cancer and HER2-positive advanced or metastatic breast cancer. Patients with stage IV-B cervical cancer ($n = 235$) were equally randomized to single-agent pazopanib (800 mg once daily), single-agent lapatinib (1500 mg once daily), or a combination of pazopanib (400 mg once daily)/lapatinib (1000 mg once daily). After treatment of 20 patients with the combination, doses were increased to pazopanib (800 mg once daily)/lapatinib (1500 mg once daily). However, the arm receiving pazopanib/lapatinib combination was discontinued at a planned interim analysis as the futility boundary for the primary PFS endpoint was crossed compared with the arm receiving single-agent lapatinib. Both median PFS and OS were significantly prolonged with pazopanib compared with lapatinib. More positive results were reported for the pazopanib/lapatinib combination in first-line treatment of patients with advanced/metastatic HER2-positive breast cancer. Patients were randomized to pazopanib (400 mg day)/lapatinib (1000 mg day, $n = 69$) or lapatinib alone (1500 mg day, $n = 72$). The 12-week progression-free rate, the primary endpoint, was lower with pazopanib/lapatinib versus lapatinib (16 vs. 37%, $P = 0.0091$) and the 12-week response rate on independent assessment was higher (36 vs. 22%). The most common adverse events of any grade occurred at similar rates in each treatment arm, but the overall grade 3/4 adverse events were more frequent in the combination arm (42 vs. 22%), and were primarily related to diarrhea (9 vs. 5%), an increase in alanine aminotransferase (12 vs 4%), hypertension (5 vs 0%), and fatigue (4 vs. 0%). Asymptomatic and symptomatic LVEF decline occurred in three and one patient, respectively, in the combination arm.

Conclusion

Although decisions on which combinations involving two or more MTTs are to be investigated clinically should be based on a strong pharmacological rationale for a particular cancer, preclinical activity studies of MTT combinations have been poorly predictive of antitumor activity and tolerability. Phase 1/2 clinical trials of MTT combinations do not show significant pharmacokinetic interactions when TKIs are combined with a monoclonal antibody. Occasional pharmacokinetic interactions have been reported when combining different TKIs. With respect to safety, such trials have usually shown that recommended doses of agents are, in general, able to be combined with some impact on increased, but manageable, toxicity, for example, skin rash, diarrhea, and fatigue. No unexpected off-target toxicity has been found. Although cardiac toxicity remains a concern, current phase 1/2 studies have not substantiated any additive toxicity.

The clinical utility of some MTT combinations has been verified in prospective, randomized phase 2/3 clinical

trials, although such studies are not generally expecting such combinations to substitute for existing chemotherapeutic or MTT/chemotherapeutic regimens. However, the combination of trastuzumab and lapatinib has shown promising results in a phase 3 trial in the first-line treatment of HER2-positive metastatic breast cancer, with superior efficacy and no additional safety problem. It is interesting to note that a combination of another anti-HER2 monoclonal antibody (pertuzumab) with trastuzumab shows activity in a randomized phase 2 trial in patients with HER2-positive metastatic breast cancer, whose disease had progressed on earlier trastuzumab-based therapy. In randomized phase 2 trials, the combination of lapatinib and pazopanib has shown additive efficacy in the treatment of first-line metastatic HER2-positive breast cancer, but not in stage IVB cervical cancer. Despite initially promising results in phase 2 study, phase 3 studies of anti-EGFR monoclonal antibodies (cetuximab or panitumumab) in combination with bevacizumab/chemotherapy in first-line treatment of patients with metastatic/advanced colorectal cancer unexpectedly showed inferior PFS and excess toxicity.

Thus, clinical investigation of MTT combinations has provided promising results in the treatment of cancer and major disappointments as well. However, the results of additional ongoing studies of different MTT combinations are still awaited and additional combinations will be tested as investigational agents progress from preclinical and early clinical investigation.

Nevertheless, it already seems that the use of MTT and their combinations, either sequential or simultaneous, will dramatically increase with important financial implications for the healthcare systems. Thus, optimization of their use through makers predictive of the expected benefit–risk is clearly needed and is indeed developing.

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Conflicts of interest

Disclosures: X. Pivot reported consultancy activities for Novartis, Roche, GlaxoSmithKline, Sanofi Aventis, and being an investigator at Novartis, Roche, GlaxoSmithKline, Bristol Myers Squibb, Pfizer, and Sanofi-aventis sponsored the trial. A. Thiery Vuillemin reported consultancy activities for Amgen, Sanofi Aventis, and being an investigator at Pfizer, Debio Pharm sponsored the trials. M. Marty reported consultancy activities for Celgene, Debio pharm, Sanofi Aventis , and has participation to the speakers' bureau of Astra-Zeneca and Roche, and being an investigator at Glaxo-SmithKline and Pierre Fabre Oncology sponsored the trial.

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