Evaluation of antiangiogenic effects: biomarkers and functional imaging

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In recent years, increasing understanding of the biology and molecular signaling pathways in cancer cells has led to the development of targeted drugs, hence enlarging the therapeutic armamentarium in otherwise chemoresistant tumors. Furthermore, targeting tumor vasculature has been shown to benefit patients with several malignancies. Antiangiogenic drugs such as bevacizumab (Avastin®, Genentech, USA), sunitinib (Sutent®, Pfizer Inc, USA), temsirolimus (Torisel®, Wyeth Pharmaceuticals Inc, USA), sorafenib (Nexavar®, Bayer, Onyx, USA) and many others have demonstrated outstanding results in a variety of cancers such as colon, kidney, lung, and liver carcinoma (Table 1).

However, questions remain on the clinical use of these drugs: identifying patients most likely to benefit from treatment, on the proper ways of monitoring response under therapy, and on mechanisms associated with resistance to those treatments.

Either plasma- and/or tumor-derived biomarkers have been proposed as predictive surrogate factors of activity and as tumor markers to monitor the biological effects of novel antiangiogenic agents.

In addition, novel imaging techniques may strengthen common Response Evaluation Criteria In Solid Tumors (RECIST) criteria, allowing to evaluate, directly in patients, the effects of drugs on tumor angiogenesis.

BIOMARKERS

Definitions

Biomarkers are defined as molecular, cellular, or functional measurable parameters indicative of a particular genetic, epigenetic, or functional status of a biological system.

Table 1. Food and Drug Administration-approved molecularly targeted therapies in solid tumors

Molecule	Target(s)	Tumor type(s)	Restriction
Imatinib	KIT, PDGF, BCR-ABL	GIST	KIT IHC +
Gefitinib	EGFR	NSCLC	
Erlotinib	EGFR	NSCLC Pancreatic cancer	
Cetuximab	EGFR	Colorectal cancer HNSCC	EGFR IHC +
Bevacizumab	VEGF	Colorectal cancer NSCLC Breast cancer	
Sorafenib	VEGFR, PDGFR, KIT, PLT-3, RAF	RCC HCC	
Sunitinib	VEGFR, PDGFR, KIT, PLT-3, RET	RCC GIST	
Panitumumab	EGFR	Colorectal cancer	EGFR IHC +
Lapatinib	EGFR, HER-2	Breast cancer	HER-2 IHC 3 + HER-2 FISH +
Temsirolimus	mTOR	RCC	
Trastuzumab	HER-2	Breast cancer	HER-2 IHC 3 + HER-2 FISH +

PDGF: platelet-derived growth factor; BCR-ABL: breakpoint cluster region-Abelson; EGFR: epidermal growth factor receptor; VEGF: vascular endothelial growth factor; mTOR: mammalian target of rapamycin; TKI: tyrosine kinase inhibitor; GIST: gastrointestinal stromal tumor; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell carcinoma; HCC: hepatocellular carcinoma; RCC: renal cell cancer; IHC: immunochemistry; FISH: fluorescence in situ hybridization.

The use of biomarkers in cancer can be helpful for diagnosis, staging, prognosis, and treatment selection. Biomarkers initially used for risk assessment and screening are also available to enhance cancer staging, refine prognosis, and estimate response to biological therapy[1].

Identifying biomarkers to evaluate response to antiangiogenic therapy is not so obvious. Indeed, they must ideally meet three conditions: (i) be easily measurable through minimally invasive procedures (for example in blood); (ii) have a prognosis value in relation with the natural course and the outcome of the disease; and (iii) have a predictive value, hence its presence correlates with the clinical response to antiangiogenic therapy.

A surrogate endpoint is an outcome measure that is a correlative indicator of clinical response or lack of response. When the surrogate endpoint is a biomarker, the outcome measure can be derived from a laboratory test that represents pharmacody-

namic markers, helping monitoring patients under therapy. To be valid, a biomarker surrogate endpoint must meet two criteria: the biomarker must correlate with the clinical outcome of interest, and must accurately capture the effect of the intervention on that outcome.

Surrogate biomarkers of drug activity are pharmacodynamic markers in which the presence or modulation correlates with the clinical response or lack of response.

These biomarkers can be divided arbitrarily in two categories: markers of exposition to antiangiogenic therapy (pharmacodynamic markers), and markers predicting the benefit of antiangiogenic therapy (predictive markers).

Markers of exposition to antiangiogenic therapy

Circulating angiogenic factors: circulating vascular endothelial growth factor receptor

Preclinical studies have shown that the evaluation of soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) may be indicative of overall circulating vascular endothelial growth factor (VEGF) levels and may be considered as a possible surrogate biomarker for VEGF-dependent tumor growth. Furthermore, an inverse relationship between the levels of sVEGFR-2 and tumor size has been observed[2].

Moreover, a significant correlation between circulating VEGF and tumor level of VEGF was shown in patients with hepatocellular carcinoma (HCC), suggesting the utility of blood levels of VEGF to evaluate the expression of tumoral VEGF[3].

Furthermore, a consistent pharmacodynamic effect of sunitinib on VEGF and sVEGFR-2 was observed in patients with renal cell carcinoma (RCC), refractory breast cancer, unresectable gastrointestinal stromal tumors (GIST), neuroendocrine tumors, and HCC. Subsequently, a significant increase in plasma levels of VEGF and a dose-dependent decrease in sVEGFR-2 levels during sunitinib treatment were observed as compared to baseline. These modifications were transient since levels returned to near baseline after two weeks off treatment[4]. Increases in plasma VEGF could result from treatment-induced hypoxia induced in the tumors[5]. Sunitinib-induced reversible increases in circulating plasma VEGF were also reported in tumor-bearing mice and, importantly, also in tumor-free mice, suggesting a systemic effect, which potentially may mask tumor-specific changes or any differences in responding patients. This observation probably involves a systemic endocrine response to VEGF and platelet-derived growth factor (PDGF) signaling inhibition in normal tissues. Furthermore, these changes in VEGF as well as the increase of placental growth factor and the decrease in sVEGFR-2 were dose-dependent and correlated with the antitumor activity of the drug[6].

Consistently, we recently observed in patients with HCC treated with sunitinib (four weeks on followed by two weeks off) a reversible increase of VEGF-A plasma levels, an irreversible decrease of VEGF-C (a ligand of the receptor VEGFR-3) and soluble KIT plasma levels and a reversible decrease of sVEGFR-2 and sVEGFR-3[7]. In the American phase II study using sunitinib 37.5 mg daily (4/2 weeks) in the same population, significant and sustained increases in VEGF, platelet-derived growth factor receptor (PDGFR) and stromal-derived factor 1 α and decreases in sVEGFR-2, sVEGFR-3, and circulating progenitor cells were also observed[8].

Likewise, these findings were observed in patients with RCC treated in the same treatment schedule. After 28 days of sunitinib, mean plasma VEGF-A level increased 2.8-fold greater than baseline and placental growth factor levels increased 3.9-fold, with a subsequent restoration to near-baseline levels at the end of the off-treatment periods. In contrast, mean sVEGFR-3 decreased by 37.6% and VEGF-C levels decreased by 22.7%[9]. These patterns suggest that the expression and secretion of these proteins may be differentially regulated by intracellular signaling mechanisms.

Regarding epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, most studies have shown no consistent correlation between EGFR protein expression by immunohistochemistry and EGFR tyrosine kinase inhibitor efficacy. Thus, EGFR protein expression has not been considered as a useful biomarker of activity of anti-EGFR tyrosine kinase inhibitors[10].

However, in a recent study, an increase in circulating ligand transforming growth factor α (TGFα) was observed upon anti-EGFR antibody but not upon receptor kinase inhibitor treatment. This feature warrants being validated in larger trials and TGFα might represent a promising potential application as a biomarker when using treatment with anti-EGFR antibodies[11].

All these biomarkers await validation in randomized phase III trials to confirm their value in predicting drug efficacy. In order to maximize efficiency in the search for valid predictive biomarkers, there is an urgent need for the standardization of their assessment, regarding the techniques used for their determination.

Circulating endothelial cells

Preclinical studies showed an increase of circulating endothelial cells and bone marrow-derived circulating endothelial cell progenitors in correlation with angiogenesis. In contrast, their levels decrease and return to normal following antiangiogenic treatments[12]. Furthermore, circulating endothelial cell levels are increased in the peripheral blood of patients affected by some types of cancer, and return to normal values in patients undergoing complete remission[12].

In clinical studies, a high dose of bevacizumab (10 mg/kg) reduced the percentage of viable circulating endothelial cells and circulating endothelial cell progenitors in some patients with rectal cancer[13]. Nevertheless, sunitinib was reported to cause a significant early , but not subsequent, increase in the peripheral blood of mature circulating endothelial cells in patients with imatinib-resistant GIST, and this observation was associated with clinical benefit (progression free survival ≥ 6 months), reflecting perhaps an increase in apoptotic endothelial cells in patients showing clinical benefit[14].

Further studies are needed to establish the potential clinical value of circulating endothelial cells and circulating endothelial cell progenitors as biomarkers of angiogenesis since the kinetic of their variation depends on the type of antiangiogenic agent and their prognostic or predictive value may be differential[15].

Moreover, the phosphorylation status of extracellular signal-regulated kinase and AKT in tumor endothelial cells has been explored as a biomarker of antiangiogenic therapy; the phosphorylation of both kinases was observed in angiogenic tumor vessels and was attenuated by sunitinib[12]. Consistently, in a phase I study of sorafenib in solid tumors, the dose level 400 mg twice daily yielded a complete inhibition of phorbol ester-stimulated extracellular signal-regulated kinase phosphorylation in peripheral blood lymphocytes[16].

Surrogates biomarkers predictive of benefit-related antiangiogenic treatment: predictive markers

Given the lack of factors available to select patients in which antiangiogenic therapy will be efficient, investigators have focused toward searching for early biomarkers of tumor response with the aim of individually tailoring these therapies.

Indeed, changes in VEGFR and VEGFR-2 plasma levels under sunitinib and sorafenib were associated with clinical benefit in patients with RCC and HCC[4,7,17,18].

More specifically, studies using sunitinib in patients with breast cancer, neuroendocrine tumors, and HCC yielded a significant increase of sVEGFR-3 similar to that of sVEGFR-2[4]. Larger decreases in VEGFR-3 plasma levels were associated with a trend for either greater estimated probability of overall survival in patients with breast cancer[19], or progression-free survival in patients with pancreatic islet cell tumors[20], raising the possibility that sVEGFR-3 may be an interesting biomarker of the effect of sunitinib on VEGF/VEGFR signaling[5]. Nevertheless, changes of sVEGFR-2 in patients with imatinib-resistant GIST treated by sunitinib were similar between patients with clinical benefit and progressive disease, suggesting that sVEGFR-2 may be useful as a pharmacodynamic marker of drug exposure but not of clinical benefit in patients with GIST[14].

Levels of soluble KIT in patients with RCC, breast cancer, or HCC also decrease significantly with the length of exposure to sunitinib[4,8,17,19]. Among patients with breast

cancer, those showing a greater reduction in soluble KIT tended to have a significantly longer time to progression ($p < 0.0001$) and overall survival ($p = 0.0194$). The same findings were observed in patients with HCC[21]. Recently, Zhu, et al. showed that decreases in plasma interleukin 6 (IL-6) and soluble KIT after 14 days of sunitinib treatment correlate significantly with improvement of progression-free and overall survival ($p < 0.05$). However, higher baseline serum levels of IL-8, IL-6, stromal-derived factor-1 and TNFα were associated with rapid tumor progression and/or mortality after sunitinib ($p < 0.05$)[8].

Interestingly, higher baseline VEGF-C levels in patients with HCC was also associated with a significant increase in time to progression and overall survival and correlated with tumor response according RECIST criteria[21]. Therefore, baseline levels of VEGF-C may be regarded as a potential predictive biomarker of sunitinib efficacy in patients with advanced HCC[21].

In contrast, lower baseline levels of sVEGFR-3 and VEGF-C were associated with longer progression-free survival and objective response rates in patients with bevacizumab-refractory RCC treated by sunitinib[9]. However, it was not known whether the association of plasma VEGF-C and VEGFR-3 with sunitinib response reported in this population was specific to the bevacizumab-refractory patients and reflective of a bevacizumab resistance mechanism, or indicative of a subset of patients who are intrinsically less responsive to sunitinib. The potential utility of plasma VEGF-C as a predictive or prognostic biomarker of sunitinib antitumor activity in HCC and RCC requires validation in larger studies.

The effects of sunitinib on soluble forms of target receptors (sVEGFR-2, sVEGFR-3 and soluble KIT) may result from a direct decrease in the number of receptor-secreting cancer and endothelial cells associated with tumor growth inhibition and/or indirect transcriptional inhibiting effects on components of VEGFR-associated signaling pathways. By contrast, the slight elevation of VEGF observed in several patients may result from an autocrine survival feedback loop trying to compensate for the reduced receptor availability[4]. Table 2 summarizes the current knowledge of potential biomarkers in patients treated with sunitinib.

Studies in which the effects of other antiangiogenic drugs, such as sorafenib, were investigated had tried to identify some prognostic biomarkers. Thus, in a phase III trial of sorafenib in patients with RCC, a higher baseline VEGF plasma level was significantly associated with poor prognosis in multivariate analysis[22]. Nonetheless, in patients with HCC treated with sorafenib, baseline VEGF plasma levels were independently associated with overall survival. Furthermore, low baseline plasma levels of hepatocyte growth factor and high plasma levels of c-KIT were independently associated with survival and with a superior response to sorafenib (HR: 1.68; $p = 0.017$ and HR: 0.56, $p = 0.003$, respectively)[18].

Table 2. Current knowledge of the potential value of VEGF, VEGFR-2, VEGFR-3, KIT and CEC plasma levels under sunitinib treatment

	Evolution under treatment	Predictive value (benefit)	Tumor type(s)	Reference
VEGF	Increase	Not demonstrated	GIST Breast cancer NET HCC	4
sVEGFR2	Decrease	No	GIST HCC	14 21
sVEGFR3	Decrease	PFS OS	NET Breast cancer	20 19
sKIT	Decrease	PFS and OS PFS and OS TTP and OS	Breast cancer RCC HCC	19 5 8, 21
CEC	Increase	PFS	GIST	14

VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor; sVEGFR: soluble vascular endothelial growth factor receptor; sKit: soluble KIT; CEC: circulating endothelial cells; PFS: progression-free survival; OS: overall survival; TTP: time to progression; GIST: gastrointestinal stroma tumor; HCC: hepatocellular carcinoma; NET: neuroendocrine tumor; RCC: renal cell carcinoma.

Further investigations in larger studies using drug-induced variations in circulating factors as surrogate biomarkers will determine whether these biomarkers can be used as indicators of efficacy, as well as their applicability to different tumor types, with different dosing regimens and during the use of combinations of antiangiogenic drugs and other agents. An important remaining question in the presence of increased circulating biomarkers is to discriminate between treatment benefit and tumor resistance or escape.

Other potential biomarkers

With an invasive approach using paired tumor biopsies from patients with GIST treated by sunitinib, Davis, et al. showed that lower baseline phosphorylated PDGFRβ in tumor biopsies was correlated with clinical benefit[23]. Moreover, under sunitinib therapy, phosphorylated PDGFRβ and sVEGFR-2 significantly decreased in patients with clinical benefit while increasing in patients with progressive disease ($p = 0.006$). Sunitinib-associated clinical benefit was also associated with significant increases in tumor and endothelial cell apoptosis ($p = 0.05$)[23].

Furthermore, in a phase II study of sorafenib in HCC, a high pretreatment tumor phosphorylated extracellular signal-regulated kinase level correlated with time to progression. In this study, 33 patients had tissue available for tumor-cell phosphorylated extracellular signal-regulated kinase staining and comparative analyses. There was a

significant difference in time to progression between patients with higher (≥ 2-4; n = 18) tumor-cell phosphorylated extracellular signal-regulated kinase staining intensity in archived specimens obtained before study treatment, versus those with lower intensity (≥ 0-1; n = 15; p = 0.00034). Patients with tumors expressing higher staining intensity had a longer time to progression. These data suggest that tumors containing higher levels of phosphorylated extracellular signal-regulated kinase may be more sensitive, or responsive, to sorafenib[24].

Recently, a correlation between the basal phosphorylation of AKT S473 and antiproliferative response to everolimus (RAD001) was observed in preclinical models[25]. Indeed, when screening the antiproliferative activity of everolimus in 13 human cancer cell lines, high basal levels of AKT S473 phosphorylation as well as the phosphorylation of the AKT substrates GSK3β and TSC2 were significantly associated with increased sensitivity to this drug. This correlation was not observed for the phosphorylation of AKT at site T308 or the AKT substrates FoxO and PRAS40. These results suggest that the epitopes GSK3β and TSC2 may be used as predictive biomarkers of everolimus activity. In contrast, no correlation between increased AKT S473 phosphorylation upon treatment with everolimus and antiproliferative response was observed in this study.

Resistance: perspective of associations

Along with the increasingly widespread use of small molecule VEGFR tyrosine kinase inhibitors and VEGF inhibitors antibodies has come evidence of the relative ease with which resistance to these agents develops when used as single agents. Thus, among patients receiving VEGFR-inhibitor therapy, and after a variable duration of treatment, some develop treatment escape resulting in progressive disease, usually from tumor rim[4].

Interestingly, in mice models a rapid regrowth of tumor vessels was shown after two days withdrawal of VEGFR tyrosine kinase inhibitors that was fully restored by seven days[26]. These results suggest a likely advantage of a continuous versus discontinuous schedule of treatment using a drug such as sunitinib[6]. In this regard, discontinuous schedules in particular may inadvertently contribute to "conditioning" of certain organ environments for metastatic tumor growth as shown in preclinical studies[27].

Actually, the mechanisms involved in resistance to VEGFR and VEGF inhibitors are hypothetical since only limited preclinical and clinical data are available. Schematically, two modes of resistance to angiogenesis inhibitors were proposed: adaptive (evasive) resistance and intrinsic (preexisting) non-responsiveness[28].

As described for KIT in GIST, EGFR in lung cancer, and breakpoint cluster region-Abelson in chronic myeloid leukemia, additional tyrosine kinase receptor mutations may lead to resistance to kinase inhibitors such as imatinib and gefitinib. Studies are

ongoing to determine whether mutation(s) of VEGFR/PDGFR or altered receptors or polymorphisms may also have a role in the resistance to antiangiogenic drugs.

A decrease in the expression level of sVEGFR has been consistently reported in patients receiving VEGFR-inhibitor therapy. Conversely, an increased level of VEGF seems to occur in many patients receiving antiangiogenic therapy and may have a role in the flare-up of tumor growth that may occur after antiangiogenic discontinuation[4].

Limited interpatient variability was observed with sunitinib in pharmacokinetic studies with target concentration levels in plasma above 50 ng/ml for activity and 100 ng/ml for toxicity. However, interindividual variability due to limited absorption capacity in some patients, variability in metabolism due to impaired liver function or cytochrome functions (sunitinib being metabolized by CYP3A4), and concomitant medications may also sometimes play a role in the under/overexposures to sunitinib, thereby reducing activity and/or increasing toxicity[29].

Other hypotheses are based on observations suggesting that activation of alternative signaling pathways may overcome VEGFR inhibition. Preclinical and pathological data corroborate clinical observations, strongly supporting the concept that VEGF is initially the main regulator of angiogenesis, and that the VEGFR-2 blockade causes vascular and tumoral regression. Nevertheless, tumor collapse also causes central regions of hypoxia that may help circumvent VEGFR-inhibited signaling pathways and promote evasion from VEGFR inhibitors in peripheral areas. In this latter phase of circumvention, which produces phenotypic resistance to VEGFR-2 blockade, tumor cells upregulate the expression of other pro-angiogenic factors (including PDGF/PDGFR and fibroblast growth factor) that reactivate angiogenesis in a VEGFR-independent manner and suggest the existence of evasive resistance[4]. Exposure to VEGFR inhibitors upregulates ephrin A1 and A2, which are primarily expressed by endothelial cells and have an important role in regulating the assembly of vascular cells into stable networks by mediating endothelial-mesenchymal cell interactions. Together, these data suggest that the combined use of other targeted therapies directed against PDGF/PDGFR, fibroblast growth factor, integrins or other kinases may help to prevent late-stage neovascularization during resistance to VEGFR tyrosine kinase inhibitors. A phase I study of sunitinib plus gefitinib in patients with metastatic RCC showed that this combination was safe and well tolerated (six out of 11 patients treated achieved a partial response and the remaining five experienced stable disease), warranting future phase II studies[30]. Another possibility would be to combine VEGFR inhibitors with targeted agents directed against kinases such as mammalian target of rapamycin, mitogen-activated protein kinases, and protein kinase C[31].

Alternatively, the resistance of this peripheral rim of viable tumor cells may be overcome by the addition of cytotoxic drugs to destroy sub-clones evading multitargeted agents. The two main endpoints of the association strategy consist of optimizing

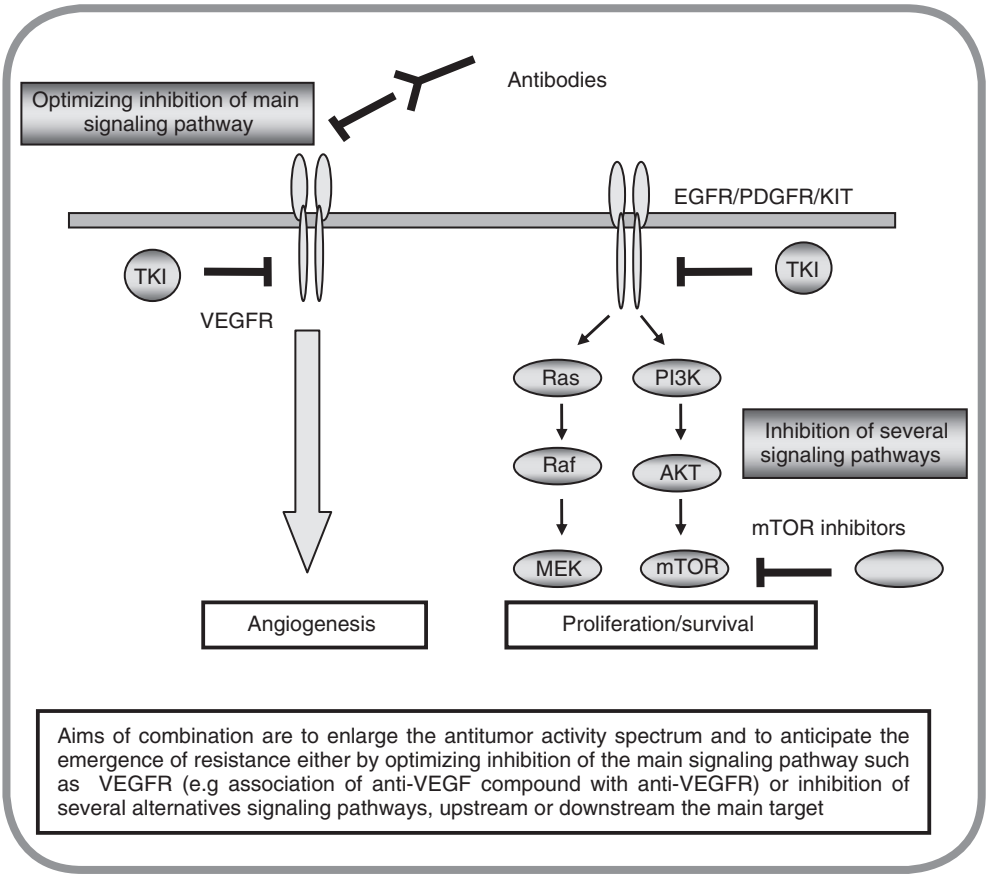


Figure 1. Potential strategies of combination of targeted therapy agents.

inhibition of the principal signaling pathway or inhibiting several alternative signaling pathways (Fig. 1). Several studies are underway combining sunitinib with gemcitabine/cisplatin in patients with non-small cell lung cancer, with paclitaxel in patients with breast cancer, and several others with docetaxel, gemcitabine, capecitabine, paclitaxel/carboplatin, and pemetrexed in patients with advanced solid tumors.

Additionally, preclinical and clinical observations have shown that vessel regression resulted in hypoxia during the course of antiangiogenic therapy and, therefore, various bone marrow-derived cells could be recruited, yielding the possibility of tumor supplement by eliciting new blood vessels and consequently providing tumors an adaptive mechanism to overcome hypoxia[28]. Moreover, it was suggested in preclinical studies that cancer cells could migrate more aggressively into normal tissue as a form of adaptation in some tumors in which angiogenesis was upset genetically and pharmacologically[28]. It was supposed that

some tumors increase the activity of a preexisting invasion program or might switch on a distinctive invasive growth program arising spontaneously during progression.

FUNCTIONAL IMAGING

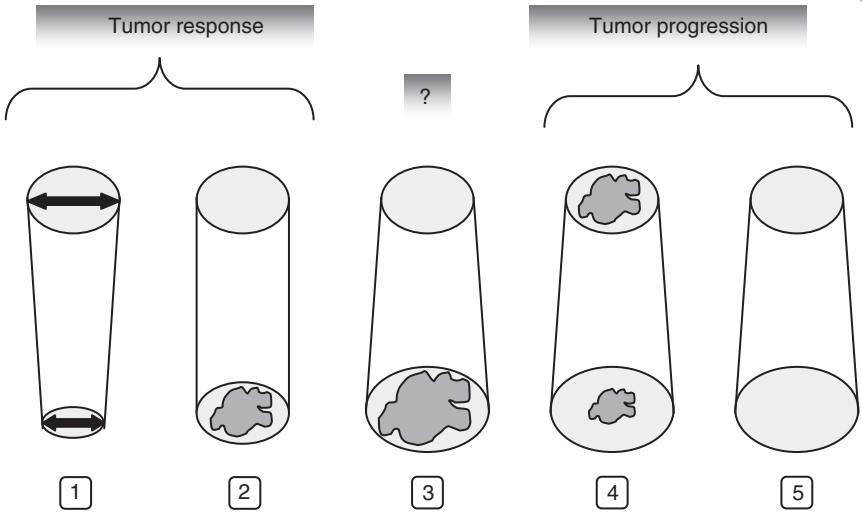
The remarkable results obtained by antiangiogenic therapies with significant survival improvement are in contrast with an objective response rate largely inferior to 20% according to RECIST criteria. This low response rate, which may have been considered as a sign of lack of antitumor activity in phase II studies, was favorably balanced by sustained tumor stabilization and minor responses, i.e. a low number of tumor progressions in the waterfall plot activity. Fortunately, the decision to proceed with phase III trial was not held back by the apparent lack of tumor response[32].

The conventional RECIST criteria usually used for tumor response evaluation compares the sum of pretreatment and posttreatment largest diameters of target lesions. With antiangiogenic therapies, a large proportion of patients do not show much modification of size of target lesions. An illustration is the case of bevacizumab in combination with conventional chemotherapy in patients with metastatic colorectal cancer that does not often increase tumor response rate, while improvement in another clinically relevant endpoint, such as overall survival, has been reported. In addition, other functional and molecular changes have been observed in tumors in response to VEGF blockade, but without a significant reduction in tumor volume[13,33].

Furthermore, some circumstances qualifying as progressive disease according to RECIST criteria might be misunderstood. A slight increase of tumor size (> 20%) can be observed despite the fact that the antiangiogenic drug may still exert an antitumor effect. In other situations, treatment-induced hypodensity of target lesions might mimic the appearance of new lesions when their visualization was not obvious at baseline (Fig. 2). Thus, in several cases the RECIST criteria appear inappropriate to evaluate antitumor activity of these new molecules.

In phase II/III studies exploring sorafenib in HCC, the objective response rate was 2.2[24] and 2.3%[34], respectively, while 39.4[24] to 71%[34] of patients had a minor response or stable disease. The same finding was observed in a phase II study with sunitinib: among 37 patients treated, only one patient (2.7%) had a partial response according to RECIST criteria[7].

Despite the lack of decrease in tumor size according to RECIST criteria, a major feature reported following VEGF and VEGFR inhibitors consists of a decreased density on computer tomography (CT) scans (Fig. 3). These morphological modifications are consistent with the antiangiogenic properties of these drugs, yielding a decrease



RECIST	Response	Stable	Progression	Progression	Progression
CHOI	Response	Response	Response	Progression	Progression

1. Partial response according to RECIST criteria defined as a decrease at least 30% in the sum of the largest diameter (LD) of target lesions.
2. Stable disease according to RECIST criteria, but response according to Choi criteria characterized by treatment-induced hypodensity ≥ 15%.
3. Progressive disease according to RECIST criteria (at least a 20% increase in the sum of the LD of target lesions), but response according to Choi since there is treatment-induced hypodensity ≥ 15% treatment-induced. The interpretation of this situation is delicate; however, it is not rare to observe survival benefit in patients receiving treatment.
4. Progressive disease according to RECIST and Choi criteria showing at least a 20% increase in the sum of the LD of target lesions and no induction of hypodensity (significant reduction of hypodensity).
5. Progressive disease according to RECIST and Choi criteria showing at least a 20% increase in the sum of the LD of target lesions and lack of hypodensity following treatment.

Figure 2. Comparison of RECIST and Choi criteria for evaluation of tumor response to antiangiogenic agents.

in intratumor vessels and blood flow in the central part of the tumor and subsequently inducing central tumor necrosis.

This feature is acknowledged to reflect the antitumor activity of VEGFR inhibitors that does not always translate into changes in the diameter of the tumor, making the radiologic evaluation of efficacy using standard RECIST criteria often inappropriate. One of the most common features is the occurrence of a rapid decrease in density in central areas of the tumor, contrasting with a sustained rim of well-vascularized tumor

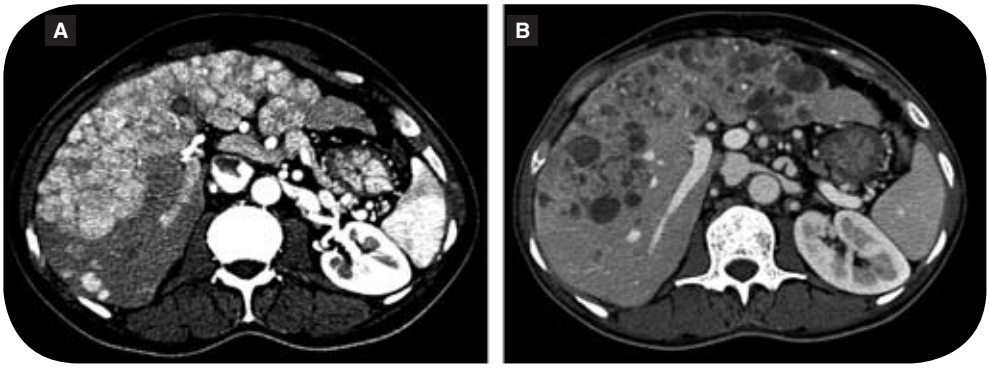


Figure 3. Example of tumor density modifications on CT scan induced by antiangiogenic therapy in a patient with advanced hepatocellular carcinoma (HCC). **A:** Imaging before treatment. Before treatment, HCC lesions appear initially hyperdense on CT scan in relation to hypervascularized features **B:** Imaging six months after treatment with sorafenib. After 6 months of antiangiogenic treatment, lesions appear hypodense and devascularized on CT scan.

tissue in the surrounding area, forming a pseudo-capsule at the interface between the tumor and normal tissue.

Tumor density rather than tumor diameter has thus been proposed as a better biometric marker of activity for clinical trials in patients receiving VEGFR-inhibitor therapy. Within the initial few weeks, hypodensity is consistent with central tumor necrosis, and is associated with lactate dehydrogenase elevation, vascular disruption, and, whenever available, a decreased level of tumor markers. In a few patients who underwent surgery for residual disease after treatment with sunitinib, pathologic examination showed a heterogeneous tumor architecture (confirming central necrosis surrounded by viable tumor cells) and undisrupted tumor vessels located in tumor margins. Interestingly, after various durations of exposure, some tumors eventually regrew from peripheral areas, suggesting that the tumor rim is the primary place of evasion from antiangiogenic VEGFR-targeting agents[4].

Beyond RECIST criteria, novel methods for evaluation of these morphologic modifications were proposed in several antiangiogenesis studies and clinical trials, such as contrast-enhanced ultrasound, dynamic contrast-enhanced MRI and dynamic contrast-enhanced CT, and positron-emission tomography[35]. Functional imaging approaches consist of intravenous injection of a contrast agent that enhances the vascular and tumoral structures and of the acquisition of sequential images before, during, and after injection. The accuracy of contrast-enhanced ultrasound for microvascular perfusion measurement and perfusion changes following therapy has been documented in patients with cancer[36].

Tumor density modifications, as determined by measuring CT attenuation coefficient, were also reported with the use of other drugs targeting KIT, PDGFR, and VEGFR such as imatinib in GIST, for which the response rate did not correlate with survival endpoints. Therefore, Choi, et al. suggest modified morphologic criteria defined by a decrease ≥ 10% of the sum of the longest diameters of target lesions and/or ≥ 15% in tumor density on CT with a sensitivity of 97% and a specificity of 100% in detecting patients with good responses[37].

When applying Choi criteria to a phase II study of sunitinib in HCC[33], 81% of patients presented hypodensity ≥ 15% of tumor mass and could be regarded as potential responders to treatment. In this study, a functional evaluation of antiangiogenic activity with dynamic contrast-enhanced CT in several patients confirmed the impact of treatment on tumor perfusion, with a rapid reduction of blood flux (median: –58.8%, range: –39.7 to –71.1%) and blood volume (median: –68.4%, range: –58.1 to –74.3%) after one month of treatment with sunitinib.

Similarly, when using sorafenib in HCC, tumor hypodensity was observed, suggesting the appearance or emphasis of tumoral necrosis. In a phase II study among 11 patients strictly evaluated for intratumoral necrosis appearance in addition to RECIST criteria, several tumors had increasing size and an increase of tumor necrosis. Before treatment, the mean diameter of these tumors was 6.4 cm (range 2.5 to 14.2 cm) and the mean proportion of tumor necrosis was 9.8% (range 0.4 to 33.5%). After treatment, the mean diameter of these tumors was 7.2 cm (range 1.7 to 16.0 cm) and the mean proportion of tumor necrosis was 27% (range 0.7 to 75%)[24].

Moreover, three years follow-up of patients with GIST treated by imatinib and monitored with contrast-enhanced ultrasound has shown an increase of contrast uptake at the first day of treatment that was predictive of future response. Furthermore, a strong correlation was found between the decline in tumor contrast uptake at days 7 and 14 and tumor response[38].

Only limited information is available trying to correlate plasma biomarkers and treatment-induced hypodensity on CT scans. In our experience, using sunitinib in patients with HCC during a phase II trial, according to RECIST criteria the baseline median level of VEGF-C was 1,523 pg/ml in patients with partial response or stable disease as compared with a baseline median level of VEGF-C of 737 pg/ml in patients with progressive disease ($p = 0.0054$)[21]. Regarding treatment-induced hypodensity, the baseline levels of VEGF-C showed a trend to correlation without reaching a significant value (baseline level of VEGF-C 1,339 vs. 815 pg/ml; $p = 0.2$), probably because of the limited number of evaluable patients in the subgroup that did not show hypodensity ($n = 4$).

Using dynamic contrast-enhanced MRI, changes in tumor blood flow following VEGFR kinase inhibitor treatment were shown in 10 patients with metastatic RCC.

Changes in blood flow at one month significantly correlated with changes in tumor size measured at four months or at time of disease progression ($p = 0.01$). Furthermore, patients with progressive disease within four months of treatment ($n = 4$) had a non-significant increase in tumor blood flow at one month ($+25 \pm 33\%$; $p = 0.43$), whereas patients with stable disease or partial response at four months ($n = 6$) had a significant decrease in tumor blood flow at one month ($-42 \pm 22\%$; $p = 0.02$)[39]. Significant decreases in vascular permeability (K^{trans}) and reverse reflux rate constant between extracellular space and plasma (K_{ep}) on dynamic contrast-enhanced MRI were also observed in patients with HCC treated with sunitinib[8]. These modifications were associated with better prognosis since the extent of decrease in K^{trans} was significantly greater in patients with partial response or stable disease compared with patients with progressive disease or those who died early following sunitinib therapy.

Interestingly, the use of dynamic functional imaging is not only limited for evaluation of tumor response, but also for early detection of secondary resistance since this has been shown in a study of enhanced-ultrasound in patients with GIST treated by imatinib. In this study, the appearance of secondary resistance was suspected by the resumption of contrast uptake of the target lesions and suggested to adjust the therapeutic strategy before observing an increase in tumor size[40].

In summary, several studies with antiangiogenic agents demonstrated the interest of additional criteria, beyond RECIST criteria, for early evaluation of antitumor activity and identification of patients who could benefit from these therapies. Further interesting and promising findings of a correlation between biomarkers and radiologic response were shown in some studies, warranting further validation in larger clinical trials.

Measurements of tumor hypodensity, intratumoral necrosis, and vascular parameters are the main criteria to be explored by dynamic functional imaging. Despite the fact that these parameters are not yet validated, they will represent prospective radiologic investigations of major interest for the assessment of antiangiogenic therapies effects beyond tumor size.

CONCLUSIONS

The success of antiangiogenic therapies in several malignancies has raised new methodologic questions in terms of evaluation criteria for tumor response, particularly in the case of stable disease according to RECIST criteria. Advances in functional imaging techniques will probably allow evaluation of these molecules in real time by assessing tumor density rather than tumor size.

Moreover, many biological and functional biomarkers of angiogenesis were evaluated in various studies. Despite none being yet validated in routine clinical use, some biomarkers are especially attractive, including VEGF-C, sVEGFR-3, and KIT plasma levels to monitor the effect of anti-VEGFR agents and to predict tumor response.

Beyond their detection and standardization, new endpoints are required from these biomarkers, such as monitoring angiogenesis, predicting response to therapy, defining the optimum biological dose, and identifying early resistance to treatment. Since sensibility and resistance to these therapies are insufficiently known, translational research will undoubtedly play a fundamental role for future optimization of these promising therapies.

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