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REVIEW

Combinations of vascular endothelial growth factor pathway inhibitors with metronomic chemotherapy: Rational and current status

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Abstract

Chemotherapy given in a metronomic manner can be administered with less adverse effects which are common with conventional schedules such as myelotoxicity and gastrointestinal toxicity and thus may be appropriate for older patients and patients with decreased performance status. Efficacy has been observed in several settings. An opportunity to improve the efficacy of metronomic schedules without significantly increasing toxicity presents with the addition of anti-angiogenic targeted treatments. These combinations rational stems from the understanding of the importance of angiogenesis in the mechanism of action of metronomic chemotherapy which may be augmented by specific targeting of the vascular endothelial growth factor (VEGF) pathway by antibodies or small tyrosine kinase inhibitors. Combinations of metronomic chemotherapy schedules with VEGF pathway targeting drugs will be discussed in this paper.

Key words: Metronomic chemotherapy; Vascular endothelial growth factor; Angiogenesis; Bevacizumab; Vascular endothelial growth factor receptor

Core tip: Metronomic chemotherapy has the potential to reduce the toxicity of chemotherapy administered with conventional schedules. In addition, understanding of the importance of angiogenesis in the mechanism of action of metronomic schedules provides a rational to combine this type of administration with targeted agents against the vascular endothelial growth factor signaling pathway.

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INTRODUCTION

Metronomic chemotherapy is defined as a chemotherapy treatment that is given more often but in lower doses than conventional chemotherapy[1]. The different administration schedule results in differences in pharmacokinetics of the given drugs and in general lower peak concentrations but more protracted trough concentrations. These pharmacokinetic differences may have implications for anti-tumor efficacy but equally importantly for adverse effects and tolerability of chemotherapy drugs.

Vascular endothelial growth factor receptors (VEGFRs) are a family of tyrosine kinase cell surface receptors that includes VEGFR-1 (also called Flt-1), VEGFR-2 (also called KDR), VEGFR-3 and the two neuropilin co-receptors NRP-1 and NRP-2. These are ligated by six secreted glucoprotein ligands VEGF-A to -E and PIGF

(Placenta Growth Factor). Ligation of the receptors triggers activation of down-stream cascades that include the Ras-Raf-MAPK and the PI3K-Akt pathways. VEGFR signaling leads to loosening of the inter-cellular junctions of endothelial cells and contributes to increased motility and eventually results in promotion of angiogenesis and vascular permeability[2]. The VEGFR system has a physiologic role during development that is usurped by tumors. Hypoxia in the tumor micro-environment is a well-known inducer of VEGF. Its expression is up-regulated by transcription factor [hypoxia-induced factor (HIF)], a factor consisting of two sub-units HIF-1α or HIF-2α and HIF-β. The β sub-unit is constitutively expressed and the α sub-units are up-regulated by hypoxia through a mechanism involving hypoxia-promoted protein stabilization. Hypoxia prevents proline hydroxylation of HIF-α factors interfering with their interaction with ubiquitin ligase (Von Hippel Lindau) and thus prevents them from being ubiquitinated and degraded in the proteasome[3]. As a result HIF-α factors concentration is increased and in association with HIF-β may initiate transcription of more than a hundred target genes, such as VEGF[4]. Other tumor associated events, such as tumor suppressor p53 inactivation and proliferation-promoting cascades activation, induce VEGF.

In this paper we will review the current data on combinations of VEGF and VEGFR inhibitors with metronomic chemotherapeutics in cancer. Drugs that affect other components of the angiogenesis network or inhibit angiogenesis indirectly may constitute rational partners for development in combination with metronomic chemotherapy but will not be discussed here.

COMMONLY USED METRONOMIC CHEMOTHERAPIES

Metronomic chemotherapy that comprises the backbone of combinations with anti-angiogenic agents is most often given in an oral form and thus availability of an oral form of various chemotherapeutics has dictated the development of such combinations, given the considerable greater ease and practicability of administering lower closely spaced in time (*e.g.,* daily) doses by mouth instead of intravenously. All these oral drugs have been developed as monotherapies or combinations with classic chemotherapy drugs previously and have a well-characterized safety profile when used in conventional doses and schedules. Data also exist for metronomic schedules, although in general less extensive than for conventional schedules. Some intravenous (IV) chemotherapy drugs given in lower doses and more frequently schedules, usually weekly, instead of three-weekly, can be considered metronomic.

Most commonly used oral chemotherapies include oral forms of cyclophosphamide, methotrexate, vinorelbine, etoposide and topotecan, the oral alkylator temozolamide and the oral fluoropyrimidines capecitabine and tegafur in combination with uracil. Metronomic oral cyclophosphamide is most commonly dosed at 50 mg per day instead of the more conventional dose of 100 mg/m^2 per day for 14 d every 4 wk incorporated, for example, in one of the versions of CMF and 600 mg/m^2 IV every 3 wk in another CMF version[5,6]. Metronomic oral methotrexate has been used often in combination with metronomic cyclophosphamide in metastatic breast cancer at a dose of 2.5 mg twice weekly as compared with the dose of 40 mg/m^2 of the classic CMF (of note this dose is considered "low" compared to "intermediate" and "high" doses in the grams range used in the treatment of sarcomas and acute leukemias and requiring folinic acid rescue)[7,8]. A usual dose of oral daily etoposide is 50 mg and a proposed dose of daily oral topotecan is 0.8 mg/m^2[9]. A commonly used metronomic dose of temozolomide is 75 mg/m^2 continuously daily compared with 150-200 mg/m^2 for 5 consecutive days every 4 wk that is a more conventional dose[10]. Of interest, the metronomic administration is part of a first line standard regimen in glioblastoma multiforme in combination with radiation therapy[10].

Metronomic administration of chemotherapy has essentially a lower incidence of some of the most serious and problematic adverse effects of conventional doses of chemotherapeutics such as myelotoxicity and serious gastrointestinal toxicity. In many cases metronomic chemotherapy produces significantly less alopecia that, although not life-threatening, is impacting in patients' quality of life.

TARGETED AGENTS AGAINST VEGF AND VEGFRS

The first anti-angiogenic agent targeting the VEGF-A ligand that entered the clinical arena was the recombinant humanized monoclonal antibody bevacizumab. Activity has been shown mainly in combination with classic chemotherapy agents in a variety of tumors such as colorectal cancer, glioblastoma multiforme, ovarian cancer and carcinomas of the lung[11-14]. Not surprisingly bevacizumab is the agent targeting the VEGF/ VEGFR axis most extensively studied also in combination with metronomic chemotherapy.

An agent targeting the VEGF ligand, that was more recently developed, is the VEGF trap aflibercept. This molecule consists of the second immunoglobulin domain of VEGFR-1, the third immunoglobulin domain of VEGFR-2 and the Fc (constant region) of IgG1 type human immunoglobulins. The construct functions as a decoy receptor that captures circulating VEGF-A and PlGF, preventing them from binding and activating their receptors[15]. Aflibercept has been approved for the treatment of metastatic colorectal carcinoma in combination with the FOLFIRI regimen[16] but has not been studied clinically in combination with metronomic schedules.

An increasing number of small molecules tyrosine kinase inhibitors (TKIs) blocking VEGFRs have gained approval for diverse indications and have entered the

clinic. They are less specific than monoclonal antibodies and tend to block other tyrosine kinase receptors and even non-receptor kinases with varying degrees of affinity. The TKI regorafenib, for example, that is approved for the third line treatment of metastatic colorectal cancer, inhibits, in addition to VEGFR, PDGF, FGFR, C-kit, Ret, BRAF and TIE-2[17]. TKIs sunitinib and pazopanib, approved for the treatment of renal cell carcinoma inhibit, in addition to VEGFR, PDGFR and C-kit, while sorafenib, another TKI also approved for the treatment of renal cell carcinoma as well as for hepatocellular carcinoma, inhibits, in addition to these three receptors, non-receptor kinase BRAF[18].

Targeted agents have a different toxicity profile from chemotherapy in general and metronomic administration in particular. A side effect peculiar to all VEGF pathway blocking agents is hypertension[19]. Skin reactions, hypothyroidism and blood coagulation complications are also other adverse effects.

RATIONAL FOR THE COMBINATION

Metronomic chemotherapy is believed to act against cancer cells in an indirect way through anti-angiogenic actions as well as through immune-mediated effects. An experimental demonstration of the importance of angiogenesis for tumor propagation in mice was obtained by using low dose metronomic cyclophosphamide. In these classic experiments metronomic cyclophosphamide inhibited angiogenesis *in vivo* and induced apoptosis of endothelial cells in a mouse experimental model[20]. This was followed by apoptosis of the human drug-resistant leukemia and lung cancer cell xenografts. Combined treatment of mice with metronomic cyclophosphamide and an angiogenesis inhibitor, TNP-470, resulted in complete eradication of drug-resistant human xenografts. Metronomic schedule consisted of cyclophosphamide 170 mg/kg every 6 d while the classic dose was 150 mg/kg in days 1, 3 and 5 every 3 wk. The effect of metronomic doses of vinblastine has been studied in human umbilical vein endothelial cells (HUVEC). Doses of 0.25-1 pM of the drug produced significant decrease in the angiogenic phenotype (proliferation, chemotaxis and adhesion to extracellular matrix components) without an increase in apoptosis of HUVEC[21]. These experiments argue for the relevance of angiogenesis in the effects of metronomic chemotherapy schedules and, in addition, offer the first evidence for the value of adding a targeted angiogenesis inhibitor.

An additional mechanism of anti-tumor action of metronomic chemotherapy is promotion of the immune response against cancer cells. In a model of tumor bearing mice, metronomic paclitaxel was demonstrated to enhance immune responses obtained with immunization with a DNA vaccine against chimeric CTGF/E7[22]. Another study of metronomic cyclophosphamide in mice bearing xenografts of glial origin or syngeneic tumors confirmed an anti-tumor activity that was dependent on recruitment of immune cells in the tumor[23]. Mice with a severe combined immunodeficiency background or defects on perforin could not mount an anti-tumor response to metronomic cyclophosphamide. In addition treatment with a more conventional intermittent schedule of cyclophosphamide produced a weaker and transient immune activation, a fact interpreted by the authors to denote a need for a more sustained immune stimulation for effective immune response production[23]. Interestingly, in this study, combined treatment with inhibitors of VEGFR axitinib, cediranib and AG-028262 and metronomic cyclophosphamide interfered with the ability of the latter to recruit immune cells to the tumors and decreased the response to it. Immune mediated activity of metronomic chemotherapy appears to be particularly dependent on doses and frequency of the used drugs, at least in pre-clinical mouse models[24]. Work from the same laboratory has shown that, in contrast to immune interference, anti-VEGFR agents may have synergistic anti-tumor effects with metronomic chemotherapy by promoting tumor retention of active metabolites such as 4-hydroxy-cyclophosphamide and, at least partially counteracting, the deleterious effect on anti-tumor immunogenicity[25]. In discordance with the above pre-clinical data arguing for a role of metronomic schedules in triggering anti-tumor immunity, a small clinical study of patients with diverse types of cancer treated with conventional or metronomic schedules of chemotherapy has reported an increase in the ratio of regulatory T cells to effector T cells in both types of schedules but this increase was more pronounced with metronomic schedules[26]. Regulatory T cells may blunt the anti-tumor response of the immune system against tumor cells. The study did not aim to compare the implications of these immune effects on clinical outcomes and, in any case, this would be impossible given the small number of patients across different tumor types and different drugs used[26]. Overall the involvement of an immune response to the anti-tumor effect of metronomic schedules of chemotherapy is far from clear but the addition of VEGF pathway-targeting agents could theoretically improve this immune effect by normalizing the tumor vasculature network and thus improving immune cell access to the tumor by preventing the formation of the pathologic tumor-associated convoluted vessel network. This is due to the fact that morphologically abnormal glomeruloid microvascular proliferations and bridged mother vessels remain dependent on VEGF-A signaling after their formation while feeder arteries, draining veins and capillaries in the tumor beds become VEGF signaling-independent[27].

PRE-CLINICAL EVALUATION

Consolidating the above rational, additional pre-clinical evaluation of combinations of metronomic chemotherapies with anti-VEGF targeted agents has been undertaken.

A human xenograft model of neuroblastoma in SCID mice was used to investigate the effect of combination

treatment of metronomic vinblastine with DC101, an anti-VEGFR2 antibody[28]. Each drug alone produced only a transient tumor inhibition. In contrast the combination resulted in sustained xenograft growth inhibition with no signs of resistance development up to seven months on treatment. In addition, some of the animals were followed after discontinuation of the combination treatment and showed no evidence of tumor recurrence. Treatment was well-tolerated and mice did not display any of the usual signs of toxicity such as weight loss, anorexia, dehydration or skin ulcerations. In agreement with the clinical effect, the combination showed more pronounced apoptotic cell death and angiogenesis inhibition than control or monotherapy treatment in histopathologic sections examination[28].

The same investigators expanded the above findings to a human orthotopic breast cancer model of cell lines MDA-MB-231 and MDA-MB-435 and multidrug resistant derivative lines expressing multidrug resistance pgp glucoprotein[29]. Continuous low dose of vinblastine, cisplatin or doxorubicin in combination with the same anti-VEGFR2 antibody resulted in improved and sustained anti-tumor effects compared with the chemotherapeutics alone while the antibody by itself had an intermediate effect.

Human colorectal cancer cell line KM12SM growing as xenograft in nude mice was investigated as a target of conventionally-dosed irinotecan or the same drug given in a metronomic schedule with or without bevacizumab[30]. Both treatments including metronomic irinotecan (with or without bevacizumab) resulted in greater anti-angiogenic effects compared with conventional schedule as measured by microvessel density and CD31 immunostaining and were better tolerated as measured by a decreased weight loss. Moreover the addition of bevacizumab appeared to act additively to metronomic irinotecan further delaying tumor growth compared with irinotecan monotherapy[30].

Another gastrointestinal tumor studied in a preclinical human xenograft model in mice is pancreatic cancer[31]. In this case the combination of metronomic schedule gemcitabine with sunitinib had specific efficacy in decreasing metastatic progression while its effect on the primary tumors was less than the effect with conventional maximal tolerated gemcitabine schedule.

Still in the realm of gastrointestinal tumor models, human hepatocellular cancer xenografts of the Hep3B cell line were studied in immunocompromised mice[32]. Treatment with sorafenib, a drug that is used clinically in the treatment of the disease, eventually led to tumor resistance and increase in tumor burden despite continuous treatment. Co-administration of a metronomic dose of UFT (15 mg/kg per day continuously) could delay the development of resistance and prolong the median survival of mice from less than 3 to 4.5 mo without increased toxicity[32]. In another study from the same group, orthotopically implanted hepatocellular carcinoma xenografts of the same cell line Hep3B were more efficiently controlled by the combination of metronomic UFT or cyclophosphamide and the anti-VEGFR2 antibody DC101 than with either agents alone[33].

In mice bearing human prostate cancer and rat gliosarcoma cell line xenografts, the VEGFR inhibitor axitinib in combination with metronomic cyclophosphamide displayed improved anti-tumor activity in comparison with either drug alone and this despite decreased tumor uptake of the active cyclophosphamide metabolite 4-hydroxy-cyclophosphamide[34,35]. Axitinib was found to possess, in addition to anti-angiogenic, direct anti-tumor cell effects inducing tumor cell apoptosis and this may relate to the fact that it is, similarly to other TKIs, an inhibitor of pathways other than VEGF, as mentioned previously[35].

Ovarian cancer was the subject of a study combining oral topotecan with pazopanib[36]. Topotecan has activity and is currently used clinically in the treatment of this type of cancer while anti-angiogenesis is also a proven beneficial modality for ovarian cancer and thus the combination is of particular clinical interest. Mice bearing human ovarian cancer xenografts in their peritoneal cavity were treated with either drug alone or their combination. Metronomic oral topotecan plus pazopanib was more effective than topotecan alone in maximal tolerated dose or pazopanib alone in reducing tumor burden and in prolonging the survival of the tumor-bearing mice[36]. Similar conclusions were reached in another study of the same combination using additional ovarian cell line xenografts[37].

In conclusion, extensive pre-clinical evidence pinpoints to the activity of metronomic chemotherapy/anti-VEGF pathway inhibitors combinations in a variety of tumor types. This together with the variety of chemotherapeutics and VEGF-targeting agents used in these combinations and producing the same synergistic effect argues for a mechanism that is independent of the specific tumor and underlying molecular lesions that it bears and is consistent with an anti-angiogenic and immune mediated mechanism.

CLINICAL STUDIES

Glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor for which the median survival with trimodality treatment followed by temozolomide maintenance is 15 mo with less than 10% of patients alive at 5 years[10]. Since several preclinical models showed up-regulation of VEGF in GBM cell lines, targeting angiogenesis has been a subject of several clinical trials that led to the approval of bevacizumab as monotherapy for recurrent GBM[38]. Few of these studies attempt to explore the role of metronomic chemotherapy in combination with angiogenic agents in this context and have attempted to bring these combinations in the forefront of GBM therapeutics.

In the front line treatment, two phase Ⅲ randomized trials showed that incorporation of bevacizumab

to the traditional radio-chemotherapy regimen with temozolomide followed by bevacizumab until progression improves the PFS, but without any benefit in OS[39]. The Radiation Therapy Oncology Group (RTOG) 0825 trial included 637 patients who were randomized to receive standard chemo-radiotherapy with temozolamide plus either placebo or bevacizumab (10 mg/kg every 2 wk)[39]. All patients had a Karnofsky Performance Scale score of at least 60. There was no statistically significant benefit of the addition of bevacizumab for the OS (15.7 mo *vs* 16.1 mo, $P = 0.21$) but a significant benefit for the PFS (10.7 mo *vs* 7.3 mo, $P = 0.007$) was noted. It is important to note that patients from the placebo group were allowed to cross over at progression, which may explain the lack of benefit in OS.

AVAglio was an industry-sponsored study conducted mainly in Europe[14]. It had a similar design that included provision to crossover on progression. It randomized 921 patients and, similarly to RTOG 0825, showed a PFS benefit for the addition of bevacizumab (10.6 mo *vs* 6.2 mo, $P < 0.001$) but failed to show an OS benefit (16.8 mo *vs* 16.7 mo, $P = 0.10$). The two trials showed contrasting results regarding quality of life (Qol) effect of bevacizumab. In the RTOG study addition of bevacizumab worsened patient neurocognitive function, while AVAglio showed improvement of Qol, prolonged time to Karnofsky Performance Scale score worsening, as well as delay in the initiation of corticosteroid treatment. One possible reason for this discrepancy between the two trials is that the AVAglio study did not incorporate a measure of neurocognitive functioning into its evaluation for Qol outcomes.

Based on the fact of failure to demonstrate benefit in terms of OS, several oncologists believe that there are not enough data to support addition of bevacizumab in front line therapy and reserve its use in large, bulky tumors with significant associated edema. Currently, Japan is the only country to approve the addition of bevacizumab in newly diagnosed GBM (http://www.roche.com/media/ media_releases/med-cor-2013-06-17.htm).

In recurrent GBM, phase Ⅱ studies have demonstrated response rates of 63% and 6 mo PFS of 38% to 46% when bevacizumab is combined with metronomic irinotecan adjusted according to whether patients were taking metabolic enzyme-inducing anticonvulsants[40,41]. In another phase Ⅱ study response rate was 47.3%, including 2 patients with complete response[42]. A phase Ⅱ trial evaluating biweekly bevacizumab with metronomic etoposide in recurrent malignant glioma[43] included 27 (of 59 total) patients with GBM. The six months PFS was 44.4%, but the toxicity was increased with the combination compared with previous reports of bevacizumab monotherapy. Interestingly low expression of VEGF in tumors assessed by immunohistochemistry was correlated with poorer PFS[43]. Another phase Ⅱ trial closed at the interim analysis as the addition of bevacizumab on metronomic chemotherapy (temozolomide or etoposide) was found to be ineffective[44].

There are limited data concerning VEGFR tyrosine kinase inhibitors in GBM. A phase Ⅱ trial has showed that the combination of sorafenib with temozolamide in recurrent GBM in 43 naïve patients for anti-angiogenic treatment is feasible and safe[45]. Twenty-three out of 43 patients had radiologically stable disease or a partial response and the median PFS was 3.2 mo and the median OS was 7.4 mo. Of note, 11% of patients suffered from a grade 3-4 hand foot syndrome.

In ovarian cancer, the activity of anti-angiogenic therapy with bevacizumab is well documented both as monotherapy (RR 21% in a phase Ⅱ study[46]) and as combination with chemotherapy. Several trials[12,47] have shown a clear benefit in PFS after incorporation of anti-angiogenesis therapy in the management of newly diagnosed advanced ovarian cancer treated with carboplatin and paclitaxel, making it a standard of care option in 1st line.

The concept of metronomic treatment with antiangiogenic therapy has been well studied in case of platinum resistant recurrent patients. A randomized phase III study comparing chemotherapy (weekly paclitaxel, weekly topotecan or liposomal doxorubicin) to chemotherapy plus bevacizumab confirmed the benefit of the combination with doubling of median PFS (6.7 mo *vs* 3.4 mo)[48]. Mature OS data presented in ESMO 2013 congress did not show statistically significant OS benefit of the combination ($P = 0.174$), despite a 3 mo survival advantage of the bevacizumab arm (13.3 mo *vs* 16.6 mo)[49]. Interestingly, in the subgroup analysis a more pronounced impact was noticed when the bevacizumab was combined with weekly paclitaxel (13.2 mo *vs* 22.4 mo), a chemotherapy regimen with anti-angiogenic activity, supporting the idea of the efficacy of the concept[49].

Moreover, a recent retrospective trial of bevacizumab and oral metronomic cyclophosphamide in heavily pretreated platinum resistant ovarian cancer, with 66 patients, has shown that the combination is active with a response rate of 42.4%[50].

The activity of weekly topotecan and biweekly bevacizumab in ovarian cancer has been shown in a trial of bevacizumab 10 mg/kg administered on days 1 and 15 and topotecan 4 mg/m^2 on days 1, 8, and 15 of a 28-d cycle until progressive disease (PD) or excessive toxicity[51]. Median PFS and OS were 7.8 and 16.6 mo respectively, with 22 of 40 (55%) of the patients being progression-free for ⩾ 6 mo. Ten (25%) patients had a partial response (PR), 14 (35%) had stable disease (SD), and 16 (40%) had PD.

A phase Ⅰ/Ⅱ study of sorafenib associated with weekly topotecan in patients with platinum-resistant ovarian cancer or primary peritoneal carcinomatosis has shown some efficacy[52]. In this study, 16 patients were enrolled in a phase Ⅰ part and 14 patients in a phase Ⅱ part. The phase Ⅱ regimen consisted of sorafenib 400 mg daily and topotecan 3.5 mg/m^2 weekly on days 1, 8, 15 of a 28 d cycle. There were 5 PR (16.7%), and 14 patients (46.7%) with SD. Nevertheless, the combination of sorafenib and topotecan caused significant toxicity.

Other studies are ongoing evaluating combinations of therapies targeting the VEGF pathway and metronomic chemotherapy in ovarian cancer. For example, a German phase Ⅰ/Ⅱ trial combines metronomic cyclophosphamide with pazopanib[53] and a phase Ⅱ study to open in United Kingdom (NCT01610869) will combine nintedanib (formerly BIBF 1120, a receptor tyrosine kinase inhibitor blocking signaling through VEGFR, PDGFR, and FGFR, also investigated in idiopathic pulmonary fibrosis[54]) and metronomic daily cyclophosphamide in patients with multiply-relapsed advanced ovarian cancer.

In breast cancer, a phase Ⅱ study of 46 patients (19 of whom had previous chemotherapy for metastatic disease) investigated the combination of metronomic cyclophosphamide at a dose of 50 mg daily with capecitabine 500 mg three times daily and bevacizumab 10 mg/kg every two weeks[55]. It showed a response rate of 48% and a clinical benefit rate of 68%. The clinical benefit was more pronounced in hormone receptors positive disease. The treatment was very well tolerated with the only grade 3 or higher adverse effect occurring in more than 10% of patients being hypertension[55]. The same investigators added erlotinib to the above combination in patients with metastatic breast cancer poorly expressing hormone receptors and negative for Her-2 and observed a response rate of 62%[56].

A phase I study with 20 metastatic breast cancer patients and up to four previous lines of treatment associated metronomic cyclophosphamide 50 mg daily with methotrexate 2.5 mg 2 d per week and vandetanib found to have a maximal tolerated dose of 200 mg daily[57]. Toxicity was acceptable and the clinical benefit rate was 25%. A similar combination with cyclophosphamide and methotrexate as the metronomic chemotherapy backbone and bevacizumab instead of vandetanib produced a clinical benefit rate of 31.8% in another study[58].

Several studies have been published showing clinical activity of metronomic schedules in advanced stage non-small cell lung cancer with acceptable toxicity profile. Data on combinations with anti-angiogenic treatments are less abundant and few studies have been published and few are ongoing. A phase Ⅱ study published only in abstract form so far presented data on the combination of two chemotherapeutics at metronomic doses (paclitaxel 80 mg/m^2 weekly three weeks out of four and gemcitabine 200-300 mg/m^2 also weekly three weeks out of four) with bevacizumab (10 mg/kg biweekly). Maintenance bevacizumab was an option for patients with a good tolerance and no progression. The trial showed a median OS of 30 mo and a 2-year OS of 55% in advanced non-squamous lung cancer[59].

A small pilot phase Ⅱ study combined a metronomic oral chemotherapy part of etoposide at 50 mg per day for 14 d of a 21 d cycle and bevacizumab at 5 mg/kg with a more intense part of cisplatin at a dose of 30 mg/m^2 for 3 consecutive days. This was followed by maintenance of erlotinib and bevacizumab in case of stable disease or response[60]. The combination demonstrated a 69% partial response rate and 86% disease control rate (partial response or stable disease). The PFS was 9.53 mo. Toxicity was also significant as expected from a regimen with a more intense component and included grade 3 or 4 myelotoxicity in 15% of patients and grade 3 or 4 GI toxicity in 18%[60].

A phase I study of the combination of metronomic vinorelbine at a starting dose of 40 mg three times per week with sorafenib (NCT00870532) has been completed but results have not been published yet.

Clinical data from studies in other tumor types, although less abundant, also confirm the concept of the combination. A study of patients with hepatocellular carcinoma and Child-Pugh class A liver function combined sorafenib with metronomic tegafur-uracil and showed a clinical benefit rate of 57%[61]. In a study of metastatic colorectal cancer patients who had at least two previous lines of therapy, the combination of cyclophosphamide 50 mg daily with imatinib 400 mg daily and bevacizumab 5 mg/kg every 2 wk was well tolerated and led to prolonged (more than 6 mo) stabilization of the disease in 20% of patients[62]. In a small study that included 15 patients with malignant neuroendocrine tumors mainly of gastrointestinal origin, the combination of temozolomide 100 mg daily with a long acting somatostatin analogue and bevacizumab produced a 64% response rate and 86% clinical benefit rate[63].

THE WAY AHEAD: PREDICTION OF RESPONSE

As witnessed by other cancer treatments such as anti-Her2 antibodies, the presence of a robust biomarker of response greatly facilitates the development and establishment of a drug in the clinic. The absence of such confirmed biomarkers for anti-VEGF treatments and for metronomic chemotherapy hampers their development in the clinic and has certainly contributed to negative results of trials in some type of cancers and moderating their success in others. Extensive investigations on discovering such biomarkers have not succeeded in bringing any biomarker forward to clinical applicability so far. Initial exploratory investigation on the predictive value of VEGF single nucleotide polymorphisms identified two minor alleles associated with bevacizumab response in a breast cancer population but this was not consistently seen in another study also in breast cancer[64]. Circulating stem or endothelial progenitor cells and MRI imaging parameters have been proposed as markers of response to sorafenib and bevacizumab[65,66]. The previously discussed report on metronomic cyclophosphamide, capecitabine and bevacizumab in breast cancer found a higher response of this treatment in patients with higher baseline circulating endothelial cells[55]. K-ras mutations, which predict for lack of response to EGFR targeting therapies in metastatic colorectal cancer, are not predictive of response to bevacizumab despite k-ras being also part of one of the intracellular signal cascades triggered by VEGFR[67]. This

argues for the importance of indirect mechanisms in the cytotoxicity of VEGF treatments. Prediction of which patient will respond to a given metronomic chemotherapy treatment is similarly currently unfeasible.

Another interesting biomarker that has been proposed as a predictor of response to bevacizumab is thrombocytosis[68]. This is a well validated laboratory value already routinely used in the clinic and has been associated with adverse outcomes in a variety of tumor types[69-72]. Interleukin-6 (IL-6), a thrombopoiesis-promoting cytokine, production by the tumor is implicated in the induction of thrombocytosis[70]. IL-6 is concomitantly an angiogenesis-promoting cytokine and thus its presence may denote a particular angiogenic propensity of tumors and dependence, as a result, to angiogenic pathways. In addition, platelets contribute to this effect by carrying additional pro-angiogenic substances in their granules[73]. As a result their number in a particular patient may represent a marker of the tumor dependency to a combination of treatments such as anti-VEGF and metronomic chemotherapy that rely on inhibition of angiogenesis as a mechanism of their action. Platelet proteome has been reported to change after treatment with metronomic cyclophosphamide, methotrexate and vandetanib[58] but it is unknown if the baseline status of this proteome predict for response to treatment. Platelet number and content represent interesting predictive markers for further investigation for the combination. Confirmation of their value and discovery of other useful predictive biomarkers will certainly facilitate a wider adoption of combinations of VEGF therapies and metronomic chemotherapy that could be a valuable option especially in later line of therapy where lower toxicity therapies are needed to fit with the profile of patients with lower performance statuses.

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