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Review Article

Metronomic therapy for gynecologic cancers

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Abstract

Systemic administration of cytotoxic drugs is the primary treatment strategy for patients with advanced cancer. The effect of cytotoxic drugs is to disrupt the DNA of the cells, rendering them unable to replicate and finally killing them; therefore, the fundamental role of a wide range of treatment regimens is typically to induce lethal toxicity in the largest possible number of cancer cells. However, these cytotoxic drugs also damage the normal cells of the host, which limits the dose of the cytotoxic drug. Thus, cancer patients are usually treated at or near the maximum tolerated dose with the implicit intent of eradicating (curing) the tumor after balancing between efficacy in tumor killing and toxicity to the host. With significantly improving patient care, most efforts are focused on the corollary, "The higher the dose, the better." However, the concept that cancer could be considered as a chronic disease and might be treated like other chronic diseases to achieve a status called tumor dormancy is gaining popularity. In addition, there has been increasing interest in putting more effort into administering cytotoxic drugs on a more continuous basis, with a much shorter break period, or none at all, and generally lower doses of various cytotoxic drugs or combinations with other newer, targeted therapies, like anti-angiogenesis agents. This practice has come to be known as metronomic chemotherapy. There is still much to be learned in this field, especially with regard to optimization of the proper drugs, dose, schedule, and tumor type applications. This review will explore recent studies that have addressed the mechanism of metronomic chemotherapy in the management of various tumors, especially gynecologic cancers.

Keywords: antiangiogenesis agent; cytotoxic drugs; gynecologic cancer; metronomic chemotherapy

Introduction

Gynecologic oncology has advanced dramatically over the past few decades, and physicians have seen the successful application of a number of conventional cytotoxic drugs to cancer conditions diagnosed in their female patients. Among these cancers, epithelial ovarian cancer (EOC) might be one of the best examples. Advances in surgical technique, anesthesia support, and intensive postoperative care significantly

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decrease the burden of the residual EOC [1]. However, surgery alone cannot treat diseases such as EOC successfully, even after aggressive surgical intervention, partly because of the presence of some residual tumor and partly because of the high recurrence of tumors; therefore, adjuvant postoperative chemotherapy with cytotoxic drugs is nearly always needed to attempt to gain complete clinical remission [2].

EOC was one of the first solid tumors to have a chance of being successfully controlled with cytotoxic drugs, although the disease-free interval is short [3]. The fundamental goal of cytotoxic drugs is typically to induce lethal toxicity in the largest possible number of tumor cells; therefore, most research efforts in chemotherapy are focused on discovery of agents and combinations of agents, doses, and dose schedules that maximally kill tumor cells while minimizing the toxicity to the host [4].

Although more than 80% of patients with advanced EOC receiving a combination of platinum and paclitaxel as primary chemotherapy initially responded, most of them developed resistant disease [5]. Only in rare cases, for example, choriocarcinoma and germ cell tumors of the ovary [6], cures can be achieved.

The use of drug regimens that have been designed to kill as many tumor cells as possible by treating with the maximum tolerated doses (MTDs) of these cytotoxic agents have been challenged in EOC recently, although dose-intensity strategy through intraperitoneal route has been reported in success. Moreover, side effects, such as neurotoxicity and damage to proliferating cells in healthy tissues pose serious constraints on the use of chemotherapy based on MTDs [7]. To allow these susceptible normal tissues like intestinal epithelium and bone marrow cells to heal, a drug-free break period before the next dose of chemotherapy is always scheduled [8]. This break period can be as lengthy as 3−4 weeks or more, depending on which cytotoxic drug or combinations are used. This practice not only involves the re-growth of tumor cells, but also growth of selected clones resistant to the agents [9]. Therefore, the patient's tolerance is believed to be one of the key factors in this kind chemotherapy. The minimal cure rate at a such a high price had made us reconsider the feasibility of MTD-based chemotherapy [10].

The bothersome side effects of cytotoxic chemotherapy include myelosuppression, hair loss, intestinal mucosa damage, nausea, vomiting, and mucositis, not to mention the long-term cardiac (for example, adriamycin), renal (for example, cisplatin), neurologic (for example, cisplatin, and paclitaxel), and reproductive (for example, cyclophosphamide) consequences, including premature ovarian failure [11,12]. Indeed, many of the recent pharmacological advances in cancer treatment involve growth factors and antinausea drugs, which are administered to cancer patients to minimize the severity of, or accelerate recovery from chemotherapy-induced toxicities, but such "supportive-care drugs" can significantly add to the financial burden of cancer chemotherapy, and have their own side effects [10].

To minimize the toxicity of MTD regimens and improve the anti-tumor effect, the future success of chemotherapy might be dependent on the integration of the metronomic scheduling of cytotoxic chemotherapy [10] and more targeted approaches [8]. Instead of only using short bursts of toxic MTD chemotherapy interspersed with long breaks to allow recovery from the harmful adverse events, there is now a shift in thinking towards the view that more compressed or accelerated schedules of drug administration using much smaller individual doses than the MTD would be more effective [13]. Much smaller individual doses separated by a short or very short interval not only reduce certain toxicities, but perhaps even improve the anti-tumor effects as well [14,15]. In addition, some of these dosing and/or scheduling strategies are ideally suited to combining chemotherapeutics with many of the new targeted and relatively non-toxic anticancer drugs that have been or are being developed [10].

A new strategy to administer cytotoxic cancer therapy

The term "metronomic" refers mainly to the scheduling, which consists of chronic, equally spaced, and generally low doses of single or combined chemotherapeutic drugs without extended drug-free breaks [9]. Chemotherapy is conventionally prescribed at the highest level that is tolerated by the patient, in order to provide the possibility of eradicating the cancer cells (Fig. 1). The evidence supporting this hypothesis is derived from the work of Skipper and colleagues [16], who utilized an *in vitro* cancer cell culture system to demonstrate the logarithmic tumor cell kill rate with increasing chemotherapeutic doses. Application of these findings to certain hematologic cancers, or "liquid tumors", such as leukemia or

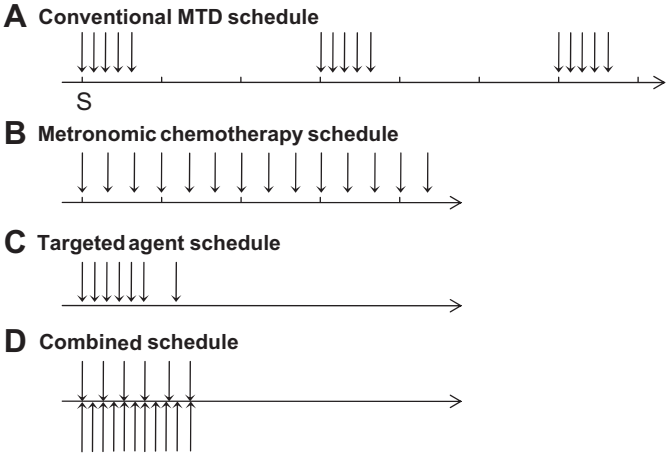

Fig. 1. Different chemotherapy regimens. Metronomic chemotherapy regimens differ from the standard MTD chemotherapy regimens that have been commonly used in medical oncology for decades. (A) In conventional chemotherapy a drug is typically given in a single bolus injection or infusion at the MTD, followed by a long break, for example, 3 weeks, before the next course of this therapy is administered; (B and C) examples of metronomic schedules are shown, in which, for example, the chemotherapy drug is administered more frequently, such as weekly (B), with no prolonged drug-free interruptions. Targeted drugs, including small molecule drugs, monoclonal antibodies, apoptosis-inducing drugs, angiogenesis inhibitors and low-dose chemotherapy, are mainly prescribed on a daily basis (C); (D) combined therapy is then ideal for the two schedules (B and C) in a chronic administration manner. MTD = maximum tolerated dose.

lymphoma, is acknowledged. However, this method might be inapplicable to the majority of solid tumors that exist within a complex microenvironment, consisting of stromal cells, immune cells, blood vessels, and many other interacting components that influence tumor biology [8,14,17].

Many factors have contributed to the emergence of new concepts in chemotherapy. First, as mentioned above, the MTD approach has failed to provide the expected survival benefits, therefore the concept of "the higher the better" has been challenged by "the more frequent the better" [18,19]. More and more evidence from randomized Phase III clinical trials has shown that 'dose-dense' chemotherapy, in which one or more chemotherapeutics are administered at more frequent intervals (that is, every other week), has clear benefits [10,20–22]. This strategy is actually designed to administer at least the same amount or, more commonly, even a greater amount of the drug, in total, over time [10,20].

Second, the concept that cancer is a chronic disease and should be treated like other chronic diseases" was proposed by Fidler and Ellis in 2000 [23]. Control of cancer was expected to be just like control of diabetes, though it might be more critical and complicated. The strategy of "treatment-for-cure" is gradually being replaced by "treatment-for-stability" to maximize the survival of victims with advanced cancers.

Furthermore, to avoid the toxicities and morbidity caused by conventional chemotherapeutic regimens and improve the life quality of cancer patients, several groups had studied a new modality of drug administration—metronomic therapy [9], a term first used by Douglas Hanahan [7], who also emphasized the concept of "less is more" instead of "the higher the better," and demonstrated the antiangiogenic effect of metronomic dosing of cytotoxic agents in mice. This schedule was then defined as a special type of treatment with chronic use of cytotoxic chemotherapeutic agents at lower and less toxic doses (Fig. 1).

Finally, a new class of chemotherapeutics called targeted therapy drugs was viewed as a potential solution for cancer treatment [24]. Since these drugs were designed to attack cancer cells using a specific marker or a particular pathway, such as angiogenesis, for cancer survival, their toxicity to the host was limited and their use could be continuous, just like the scheduled application in metronomic chemotherapy (Fig. 1). The combination of targeted therapy and metronomic chemotherapy was believed to be more effective in advanced cancers [25], and yield a better survival rate (Fig. 1).

Mechanism of metronomic therapy

Although the exact mechanism remains unclear, the reason why metronomic chemotherapy works might be vasculature-related and based on a concept proposed by Judah Folkman and Robert Kerbel [25,26], who were pioneers in making this proposal for a change in the way with which chemotherapy is undertaken. In 1971, Folkman [27] first articulated the concept of what he called "antiangiogenic" agents. Based on observations that expansion of a tumor mass was limited in the absence of angiogenesis, he proposed that treatment with drugs that prevent the formation of tumor blood vessels might be able to constrain or starve cancer cells eventually [27]. Kerbel's group [10] further demonstrated the antitumor efficacy of using some of the most widely used cytotoxic drugs. When given continuously in low doses, these agents showed anti-angiogenic effects, and were a promising therapeutic choice for treating tumors that no longer responded to conventional chemotherapy [10] with limited or no toxicity [28].

The success of metronomic therapy is currently believed to rely on three main mechanisms: continuous administration, activation of cancer immunology, and antiangiogenic effects [8]. First, tumor cells could be exposed to the drug more continuously by diminishing the drug-free period that accompanies MTD regimens. As in radiation therapy, a continuous low-dose schedule might permit more efficient cancer cell destruction as cells continue to proliferate or enter into the growth cycle [8]. There would be significantly less opportunity for repair of the damaged endothelial cells or tumor cells and the anti-tumor effects of the chemotherapy would irreversibly accumulate in a metronomic regimen.

Second, the tumor escaping from the surveillance of host immunity might play a role in cancer development [29]. Instead of the deleterious myelosuppression induced by MTD chemotherapy, certain cytotoxic drugs in metronomic schedules display immune-stimulatory effects that can enhance the immune response to cancer antigens [30].

Finally, and most importantly, inhibition of tumor angiogenesis was demonstrated to be the main effect of metronomic chemotherapy [31]. Angiogenesis is the process of establishing a new circulation system, which involves the recruitment of sprouting vessels from existing blood vessels and incorporation of endothelial progenitors into the growing vasculature [32]. This process of new blood vessel growth that occurs in a variety of physiologic and pathophysiologic states [33–36] not only brings forth oxygen and nutrients, but also removes wastes. A prosperous blood supply network is fundamental for a solid tumor to grow beyond several millimeters in diameter [8]. These "activated" endothelial cells of newly forming capillaries in cancer demonstrated high and selective sensitivity to very low doses of various chemotherapeutic agents *in vitro* [10]. Several studies have used very low concentrations of cytotoxic drugs, such as vinblastine [37, injectable taxanes [38], and paclitaxel [39] to test various human cells, including microvascular and macrovascular endothelial cells, and found that these drugs inhibited the proliferation or migration of endothelial cells, but not of other cell types that were examined.

These continuous chemotherapeutic schedules have had effects on at least four specific aspects of angiogenesis (Fig. 2). The first is the direct cytotoxic effect on the endothelial cells of the blood vessels (Fig. 2A). With an outcome not possibly achieved with a conventional schedule, Browder and colleagues [40] demonstrated that metronomic use of cyclophosphamide suppressed certain tumor growth threefold more effectively. Though it is not so easy to differentiate the detrimental effects between the tumor population and angiogenic progenitor cell population, which mix in general homogenously or heterogeneously, and it is relatively simple

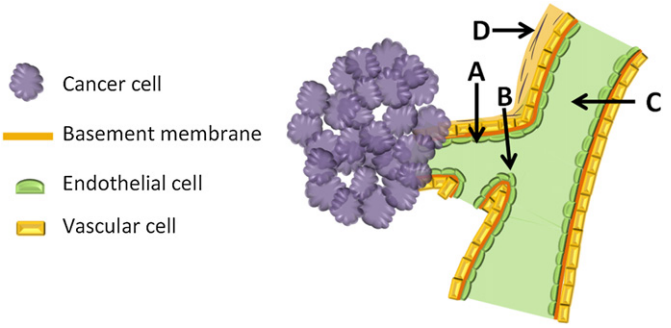

Fig. 2. Four specific aspects of angiogenesis have been shown to be impacted by these continuous cytotoxic chemotherapeutic schedules, including (A) direct damage to endothelial cells; (B) alteration of the balance of growth factors; (C) decrease in the progenitor of endothelial cells from bone marrow; (D) and inhibition of MMPs. MMP = matrix metalloproteinase.

to study them *in vitro* [40], a detailed temporal analysis showed that the endothelial cells were the first part of the tumor to undergo apoptosis [26].

The second involves decreasing angiogenesis by an indirect effect, which is accomplished by a new balance of growth factors related to angiogenesis that hinder vessel expansion (Fig. 2B). These events might involve the inhibition of growth factors like vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and cyclooxygenase-2 (COX-2), or damage to relevant receptors. Bocci's group [41] found that selective inhibition of human endothelial-cell proliferation or induction of apoptosis was detected only after a prolonged exposure to very low concentrations of a number of different chemotherapeutic drugs, including taxanes and alkylating agents. This delay showed that the cytotoxic effects of low-dose metronomic chemotherapy on endothelial cells might not be direct, but could be a secondary result of some other process that is specific to the vascular endothelial cell [10].

Third, the expansion of tumor blood vessels may also rely on, or at least be influenced by, recruitment of bone marrow–derived endothelial progenitor cells [8]. A decrease in the levels and viability of endothelial progenitor cells was documented after metronomic chemotherapy [42]. Adverse effects on these cells resulted in decreased angiogenesis (Fig. 2C).

Finally, the action of matrix metalloproteinases (MMPs), which are enzymes that break down extracellular matrix to allow room for migration of endothelial cells to form blood vessels, may also be blocked by these agents [34]. This will also limit new vessel development and further expansion of the cancer (Fig. 2D).

Metronomic therapy used in gynecologic cancers

Several metronomic regimens of cytotoxic agents were used to rescue patients with advanced or refractory gynecologic cancers. Metronomic use of chemotherapy was mainly focused on recurrent EOC, however, high fractions and low dose-rate radiotherapy for advanced cervical cancer was viewed as metronomic radiotherapy and included below.

Epithelial ovarian cancer

Over the past few decades, a number of conventional cytotoxic drugs have been successfully used with EOC patients. Although adjuvant postoperative chemotherapy with cytotoxic drugs allows nearly all patients with EOC to achieve complete clinical remission [2], the disease-free interval is short and often transient [3]. In addition, the high rate of resistance and the massive toxicity of this regimen pave the way for a metronomic regimen.

Cyclophosphamide

The antiangiogenic action of metronomic use of this agent was first demonstrated in a murine model of cyclophosphamide-resistant tumors designed to rescue mice by inducing endothelial apoptosis [26]. Metronomic dosage of oral CTX (20 mg/kg/day), combined with injected irinotecan (10 mg/kg, twice a week), cisplatin (1 mg/kg, twice a week) or paclitaxel (1 mg/kg, three times a week) in mice was proved to be safe, though not very effective in this mice model [43].

However, the use of low-dose oral CTX was recommended as a potential strategy against tumor progression in platinum-resistant patients with resistant EOC after standard chemotherapy and with a poor performance status [44].

Metronomic therapy using low-dose CTX could be combined with oral 5-FU [45] or an antiangiogenic drug, bevacizumab (10 mg/kg intravenously, every 2 weeks), and proved to be safe and effective in recurrent EOC patients [46], even in patients with failed chemotherapy using cisplatin, gemcitabine, topotecan and liposomal doxorubicin chemotherapy [47,48].

Topotecan

A dosage of 1.5 mg/M^2 infusion for more than 30 minutes on 5 consecutive days every 5 weeks is recommended in a MTD regimen for ovarian cancer. A continuous low dose of irinotecan (10 mg/kg) was proved to be safe and effective in a mice model of ovarian cancer [43]. A metronomic regimen of topotecan (1.0 mg $\pm$ 0.5 mg once every day (qd)) showed promise in an advanced EOC cell model through its anti-vascular effect [49]. Oral topotecan (25 mg/day twice every day (bid) or 100 mg/kg/day) has demonstrated a positive effect against ovarian cancer in mice, especially when combined with an anti-angiogenic drug, pazopanib (150 mg/kg/d) [43]. However, a high drop-out rate (6/13) was noted in a test using oral topotecan with a metronomic dosage as low as 0.3 mg/m^2 twice daily for 21 consecutive days out of 28. Severe side effects like hematologic toxicity and diarrhea still occurred in these patients with advanced EOC. The appropriate dose and schedule of this agent used in a metronomic regimen for humans are still worth studying [50].

Taxanes

The usual dosage of paclitaxel as an adjuvant chemotherapy for EOC is 135 mg/M^2 every 3 weeks. Lower-dose

paclitaxel (1 mg/kg, three times a week) was used in a metronomic combination in a mice ovarian cancer model, with minimal toxicity and a potential effect [43]. Metronomic taxanes (0.5 mg/kg, twice weekly) alone and in combination with oral AEE788 (50 mg/kg, twice weekly)—a dual EGF receptor (EGFR) and VEGF receptor (VEGFR) inhibitor—were used in an orthotopic mouse model of EOC and also showed promising effectiveness [51].

Cisplatin

A lower dose of cisplatin (1 mg/kg, twice weekly) was used in combination with other cytotoxic agents in mice with proved safety and efficiency [43]. Since platinum was usually included in the MTD chemotherapy regimen (100–200 mg/M^2, 2–6 hours every 3–4 weeks) as the first-line adjuvant therapy for ovarian cancer, the fact that there are limited reports about this regimen for recurrent ovarian cancer is reasonable. However, metronomic cisplatin still revealed anti-angiogenic and anti-tumor effects in a rat model [52], and has been used with other cancers like colon or lung cancer.

5-fluorouracil (5-FU)

Metronomic use of this agent (24–120 mg/kg/week) showed an antiangiogenic effect and resulted in body weight increase in a rat model [52]. An oral 5-FU prodrug (UFT), when used in a metronomic way and combined with oral CTX and bevacizumab, an anti-VEGF monoclonal antibody, showed encouraging results in recurrent ovarian cancers [45].

Uterine cancer

There is a limited amount of literature on metronomic therapy focusing on uterine sarcoma. Metronomic doxifluridine alone significantly suppressed the growth of human uterine carcinosarcoma cells and reduced intratumoral vascularity, while metronomic doxifluridine in combination with TNP-470 significantly inhibited tumor growth and vascularity compared with each treatment alone [53]. Metronomic irinotecan (4 mg/day) combined with low-intensity ultrasound (2.0 w/cm^2, 1 MHz) for 4 minutes three times per week showed more potential effect in the treatment of human uterine sarcoma than each treatment alone [54].

Metronomic scheduling of cytotoxic drugs, when combined with imatinib mesylate (Gleevec; Novartis Pharmaceuticals Corp., East Hanover, NJ, USA) showed a promising effect on uterine malignancy [55].

Cervical cancer

If we view radiotherapy as an agent for cancer treatment, high fractions and a low dose rate (LDR) schedule for this therapy are the origins of the development of the metronomic regimen. As such, the changing course of radiotherapy for cervical cancer demands discussion. Compared with EOC, squamous cell carcinoma of the uterine cervix is more sensitive to radiotherapy, and it has been the treatment of choice for local advanced cases.

To diminish the local hazard effect and increase the accumulated dosage, several LDR schedules with some modification, like pulsed brachytherapy [56] or a continuous LDR remote after-loading brachytherapy using the Martinez Universal Perineal Interstitial Template (MUPIT) [57] have been proposed to achieve fewer adverse events and better results. Wide ranges of dose rates, from 0.1 cGy/day to several Gy/minute, have been tested and the equivalent effect of high and LDR intracavity treatment for carcinoma of the uterine cervix seemed supported, though several questions remained to be answered [58]. In addition, more efficient inactivation of cervical cancer cells at lower (0.33 Gy/hour) rather than at higher dose rates (0.86 Gy/hour) was observed for radiation doses exceeding 7 Gy, due to the pre-mitotic accumulation of cells during LDR irradiation [59].

As for chemotherapeutics used in cervical cancer, platinum (a continuous low-dose of intra-arterial infusion cisplatin [60] or a continuous infusion of carboplatin (12 mg/m^2/day) [61], considered to be a radiosensitizer, is the most active single cytotoxic agent [60]. A continuous LDR of 5 cGy/minute instead of a higher dose rate of 70 cGy/minute when combined with four agents, including bleomycin, etoposide, cisplatin, and ifosfamide, led to substantial additional growth delay in a mice model [62]. Furthermore, different RT regimens were combined with chemotherapy to increase tumor radiosensitivity. Hyperfractionated radiotherapy (HFRT, 1.2 Gy in two daily fractions, totally 60 Gy) with concurrent chemotherapy (MTD of paclitaxel or cisplatin, weekly) for para-aortic lymph node (PALN) recurrence of cervical carcinoma was proved to be effective and safe [63].

However, since concurrent platinum-based (cisplatin, 40 mg/m^2, weekly) [64] chemoradiation has become the standard treatment of choice for locally advanced cervical cancer patients, even for those after LARVH (laparoscopically assisted radical vaginal hysterectomy) [65], many recurrent tumors may therefore be platinum-resistant [66]. And other synergic drugs like Aurora kinase B inhibitor (ZM447439) [67], 5-FU (continuous infusion 750 mg/m^2, for 5 days every 2 weeks) [68], a prolonged regimen of 5-FU (1000 mg/m^2, 24-hour continuous intravenous infusion, 4 days every 4 weeks) [69], the polyADP-ribosylation inhibitor, 3-aminobenzamide, (3-AB, administered at 450 mg/kg) [70], a biweekly PEB regimen (100 mg/m^2 etoposide + 50 mg/m^2 cisplatin + 50 mg/m^2 bleomycin) [71], weekly PEBF (50 mg/m^2 etoposide + 20 mg/m^2 cisplatin + 10 mg/m^2 bleomycin + 800 mg/m^2 5-FU, mixed in normal saline, 24-hr continuous iv infusion) [71], and the use of retinoids and interferon [72], enhance the radio-sensitivity of cervical cancer.

Targeted therapy used in combination with metronomic therapy in gynecologic cancers

To enhance the success of metronomic therapy in the management of cancers, other adjunctive drugs targeting the processes that transform healthy cells into malignant tumors

[24] or different parts of the cancer microenvironment [33] have been taken into consideration. A new class of drugs called targeted therapies, including small molecule drugs, monoclonal antibodies, apoptosis-inducing drugs, angiogenesis inhibitors, and cancer vaccines [24] has been under investigation for adjuvant use in the treatment of various advanced gynecologic cancers. In fact, the approval of various antiangiogenic therapeutics for the management of different advanced malignancy is one of the most significant achievement in medical oncology practice [45]. A metronomic schedule made the combination of traditional chemotherapeutics and these targeted drugs in a continuous and complementary way and might be proved to be the most effective regimen [73].

Targeted agents with anti-angiogenic effects may provide an improvement in the treatment of patients with recurrent ovarian cancer and may be useful when incorporated into first-line platinum/taxane therapy [74]. Though the efforts put into finding a single targeted agent for the treatment of cancer never stops, these agents, when used alone, demonstrated only modest efficacy in treating cancer [75,76]. Therefore, the benefit of these agents may still lie in their ability to combine safely and efficiently with chemotherapy [76] or radiotherapy [31]. A detailed review of these targeted agents used in gynecologic cancers can be found elsewhere [77]. However, there are a limited number of agents used in combination with metronomic cytotoxic drugs in gynecologic cancers, and these agents still mainly act on the angiogenesis process. Strategies devised to inhibit angiogenesis of malignant tumors include neutralization of circulating growth factors, direct inhibition to receptors, blocking growth factor production, inhibition of ripped pocket (RTK) activation and suppression of intracellular signaling cascades [31]. The drugs in this group can be categorized accordingly (Fig. 3) [78].

The VEGF family is comprised of six secreted glycoproteins, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor [79]. The best-characterized VEGF family member is VEGF-A (commonly referred to as VEGF. VEGF status is significantly correlated with neovascularization grade and prognosis in various types of solid tumors [80] and serves as a predictor of tumor resistance to various treatments, including radiotherapy and chemotherapy [36]. Binding of VEGF prevents angiogenesis (Fig. 3A).

The biologic activity of VEGF is dependent on its reaction with specific tyrosine-kinase receptors called VEGFR [80]. These receptors consist of 3 located on cell membranes and VEGFR-2, which is predominantly localized on vascular endothelial cells [81], and mediate the majority of downstream signal transduction, including the nitrous monoxide pathway [82] and eventually angiogenesis [79]. Direct damage to the receptor (Fig. 3B), inhibition of tyrosine kinases (Fig. 3C), or blockage of the following signal transduction pathway (Fig. 3D), all have an antiangiogenic effect.

VEGF is produced mainly by malignant cells in response to events like hypoxia or inflammation, and is stimulated by substances like growth factors or cytokines, e.g., tumor necrosis factor alpha (TNF-α), angiopoietin-1 and angiopoietin-2, interleukin-8 (IL-8) [37,83]. These

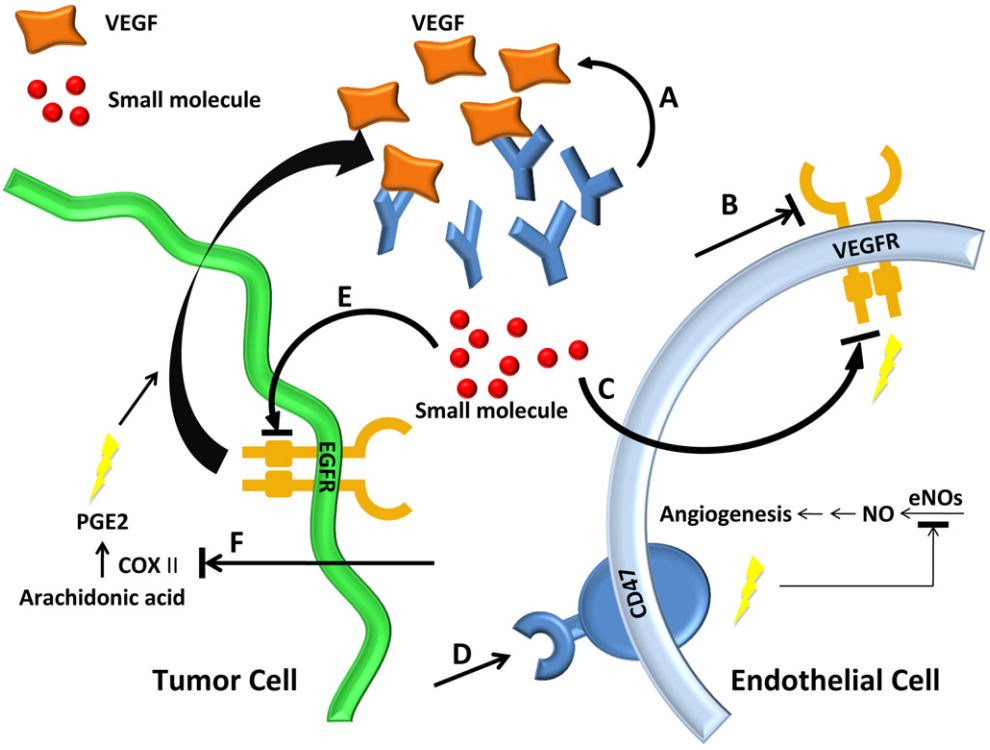

Fig. 3. The specific targets of antiangiogenesis targeted agents. Strategies devised for targeted adjuvant treatment to inhibit angiogenesis of malignant tumors include (A) neutralization of circulating growth factors with monoclonal antibodies; (B) direct inhibition of VEGFR; (C) inhibition of RTK activation of VEGFR with small molecular drugs; (D) blockage of NO-related signal pathways of angiogenesis; (E) and antagonizing the stimulators of VEGF via inhibition of EGF; (F) or following signal transduction. EGF = epidermal growth factor; NO = nitric monoxide; RTK = ; VEGFR = vascular endothelial growth factor receptor.

stimulators, including growth factors like EGF and PDG F-β [36], execute their actions by binding to receptors on tumor cells. Inhibition of this cascade (Fig. 3E) or relative transduction pathway (Fig. 3F) will result in a decrease of VEGF, and the following angiogenesis.

These targeted agents are still mainly used in advanced ovarian cancers as an adjunctive regimen of metronomic chemotherapy. Only sporadic trials have focused on recurrent uterine cancer and cervical cancer.

Epithelial ovarian cancer

Most of the targeted agents are evaluated first in the treatment of ovarian cancers, since metronomic schedules of cytotoxic agents are mainly devised for the treatment of recurrent ovarian cancers.

Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody that neutralizes VEGF (Fig. 3A). In 2004, it became the first drug approved solely as an angiogenesis inhibitor, originally for the treatment of colorectal cancer and later for other cancers [84]. It is also the drug used in combination with metronomic chemotherapy that has achieved the most striking results so far.

It has demonstrated a progression-free survival benefit in combination with first-line paclitaxel/carboplatin and as maintenance therapy in EOC [85]. It was also used as a single agent or in combination with cytotoxic drugs in the treatment of recurrent EOC patients, yielding an increased response rate and more months of survival [86]. A dosage of 15 mg/kg every 3 weeks also showed an improved clinical response in patients [87]. Several studies have illustrated the feasibility of combination treatment utilizing bevacizumab with a variety of cytotoxic agents, like oral CTX [88], gemcitabine [89] and platinum [89], although some tolerable hematologic toxicities occurred [89].

Unique toxicities like proteinuria, hypertension, bleeding, thromboembolism, wound healing complications, and GI perforation or fistula have been ascribed to the administration of bevacizumab and other anti-VEGF molecules for patients with advanced malignancy. The former 3 toxicities were generally mild and controllable; the later three are serious, though uncommon [32].

All the data summarized above support the use of bevacizumab, an anti-angiogenic drug, as a single or one of a combination of agents in the metronomic treatment of gynecologic cancer.

Thalidomide

Thalidomide is an immune modulator acting on VEGFR to inhibit angiogenesis (Fig. 1B). It has been investigated extensively in the treatment of advanced cancers. Significant activity in multiple myeloma and myelofibrosis syndrome has been confirmed. A less prominent effect on solid tumors like Kaposi sarcoma, malignant melanoma, renal cell carcinoma and prostate cancer has been demonstrated, especially in combination with chemotherapy [90]. There was only limited activity or even no effect in certain groups of advanced EOC when daily dosages of 200–400 mg were used alone [91] or in combination with tamoxifen [92].

On the other hand, the addition of thalidomide (200 mg daily) to topotecan for the treatment of recurrent ovarian cancer was proved to improve response rates, and is recommended for phase three trials [93]. Furthermore, daily thalidomide used as a conjunctive of weekly topotecan (a metronomic schedule) was studied and accepted as a reasonable alternative to standard MTD topotecan chemotherapy [94].

Vandetanib

EGFR inhibitors are a new biologically targeted therapy that may offer new hope in the treatment of patients with advanced or recurrent ovarian cancers [76]. As a small-molecule receptor tyrosine kinase inhibitor, vandetanib acts on both VEGFR and EGFR [95], resulting in the diminishing of both angiogenesis (Figs. 3C and E) and tumor growth [96]. Its use in dosages of 50 mg/kg daily was proved to be active in a mice ovarian cancer model when used alone [95] or combined with daily paclitaxel 20 mg/kg intravenously [97]. Though vandetanib 300 mg daily monotherapy had no significant clinical benefit in some settings of recurrent EOC, and only tolerable adverse effects like rash, diarrhea, and a prolonged QT interval, no hypertension was encountered and its safety in the same setting has been confirmed [75]. More clinical study of this agent in combination with cytotoxic agents will clarify its effect as an adjunctive therapy in recurrent EOC. Two other similar agents should be mentioned here, pazopanib and imatinib.

As another multireceptor tyrosine kinase inhibitor, pazopanib can block the function of both VEGFR and EGFR (Figs. 3C and E). In contrast to vandetanib, the combined use of this agent with metronomic chemotherapy has been evaluated in mice, and its use (25 mg/kg twice a day or 150 mg/kg daily) in combination with oral metronomic topotecan (1 mg/kg daily) showed an excellent anti-tumor effect in an ovarian cancer cell line by reducing the activity of VEGFR-2 in a dose-dependent manner [73]. Similar efficacy was found with the combination of metronomic docetaxel chemotherapy (0.5 mg/kg three times a week) in dual EGFR and VEGFR inhibition [51].

Imatinib mesylate is a tyrosine kinase inhibitor (Fig. 3E) with activity against abl, c-kit, and PDGF receptor (PDGFR). It is not only approved for the treatment of some leukemia and gastrointestinal stromal tumors, but is also used with gynecologic malignancies with a promising effect either as a single agent or in combination with metronomic scheduling of cytotoxic drugs [55].

Thrombospondin-1

Another possible antiangiogenic choice is inhibition of the nitric monoxide (NO) signaling pathway (Fig. 3D). NO induces several important physical functions including increased vessel diameter, decreased platelet aggregation,

elevated perfusion, and angiogenesis. Experiments focusing on endogenous NO synthase (eNOS), the primary endogenous source of NO in endothelial cells, demonstrated a deficiency in both angiogenesis and permeability response to VEGF in eNOS-null mice [82]. Therefore, blockage of eNOS has been considered a promising way to inhibit angiogenesis and tumor growth.

Thrombospondin (TSP), the first identified endogenous angiogenesis inhibitor, also controls tissue perfusion, hemostasis and radiosensitivity by antagonizing NO signaling [98]. The TSP family comprises five secreted proteins that have distinct roles in development and physiology [98]. Among these secreted proteins, TSP1 is considered a potent angiogenesis inhibitor. The current Food and Drug Administration-approved angiogenesis inhibitors, such as ABT-510, a peptide sequence in the anti-angiogenic type 1 repeats of TSP1, are expected to inhibit eNOS activation and result in both decreased NO and angiogenesis.

ABT-510, a promising candidate for the inhibition of tumor growth, was proved to induce tumor cell apoptosis in mouse and human ovarian cancer cells [99]. Furthermore, the regimen of ABT-510 (100 mg/kg daily) in combination with cisplatin (2 mg/kg per 3 days) or paclitaxel (10 mg/kg every other day) resulted in a significant increase in the apoptosis of tumor cells and tumor endothelial cells, and then a decrease in ovarian tumor size when compared with the use of these agents alone in a EOC mice model. Through activity on the tumor vessels, ABT-510 increases the uptake of chemotherapy drugs and induces the regression of advanced EOC [100].

However, inhibition of the NO pathway may account for the hypertensive and thrombus adverse events of the VEGF antagonists; a new TSP analogue that works through the CD47 receptor selectively may prevent these side effects [98].

Celecoxib

COX-2 overexpression is associated with poor outcome and resistance to platinum-based chemotherapy in ovarian cancer. As a COX-2 inhibitor, celecoxib is believed to decrease VEGF [101] by blocking the related pathway in tumor cells (Fig. 3F). Inhibition of COX-2 may also diminish lymph node metastasis by suppressing VEGF-C-mediated lymph angiogenesis [102]. Celecoxib (400 mg daily) was used in combination with intravenous carboplatin in recurrent heavily treated EOC patients with promising activity and tolerable toxicity [103].

In ovarian cancer cell lines, exposure to celecoxib alone inhibited cellular growth and proliferation in a dose-dependent manner [104], and when used in combination with cisplatin, celecoxib demonstrated a clearly antagonistic effect that was independent of COX-2 expression levels [105]. Other apoptotic effects of celecoxib on tumor cells might be irrelevant to the COX-2 pathway.

The use of celecoxib as a preventive drug for ovarian cancer is currently being evaluated and has achieved a positive result, so far [101], and promising results can be expected, especially when celecoxib is used in combination with metronomic cytotoxic agents.

Hormone therapy

Endocrine therapy is a unique and recognized option in the treatment of chemoresistant ovarian cancer. Synergistic and additive activities were demonstrated when using tamoxifen (TAM) combined with cisplatin, doxorubicin, or CTX [106]. Combination endocrine therapy with tamoxifen 20 mg twice daily and goserelin 3.6 mg subcutaneously once a month was proved to be an active regimen with minimal toxicity in platinum-resistant ovarian cancer patients [107].

Multitargeted agent regimens

A regimen using a combination of thalidomide, imatinib and celecoxib for up to 24 months following initial chemotherapy exhibited excellent results in sporadic young advanced ovarian cancer victims [108]. It remains to be determined whether multi-targeted agents or combined agents with different targets will offer greater clinical benefit than specific and single VEGF pathway inhibitors [74].

Uterine cancer

Unlike the case with EOC, the literature on targeted agents used in uterine malignancy is sparse. Thalidomide was once used, but no activity in patients with uterine leiomyosarcoma was demonstrated, nor was any alteration in the VEGF concentration documented [109]. Vandetanib has shown anti-tumor effects in a human uterine sarcoma mice model, and the adverse effects on the tumor-associated microenvironment have also been demonstrated [110]. However, only a modest partial response rate was verified when bevacizumab was used as the only agent in recurrent uterine cancers [111].

Cervical cancer

Loco-regional control continues to be the main goal in the treatment of local advanced cervical cancer [112]. Therefore, targeted agents seem to play the role of radio-sensitizer, similar to the metronomic cisplatin regimen. The use of celecoxib in this regard has gained the most attention. Celecoxib at 400 mg twice daily with concurrent cisplatin, 5-FU MTD chemotherapy and pelvic radiotherapy presented increased efficacy but a high incidence of acute toxicities [113] and late complications [114], which hindered its use for advanced cervix cancer and paved the way for the combination regimen of target agents and metronomic chemotherapeutics.

The use of bevacizumab as a single agent [115] or in combination with cytotoxic drugs was proved to be not only active but also safe in treating recurrent cervical cancer [116]. However, the identification of more active biologically targeted therapies is warranted for the treatment of advanced cancer of the cervix [112].

Unsolved problems

Although gynecologic oncologists pay an attention in the use of metronomic therapy in cancer treatment, there are still many

unresolved problems regarding its use. First, the key factor of the success of this regimen might be the anti-angiogenic effect of the continuous use of cytotoxic agents, but the optimal therapeutic dose is still unknown. The main reason is that we do not have enough evidence to determine the 'individualized maximum repeatable dose' of every drug we use for circulating endothelial (progenitor) cells [117]. Second, the role of targeted agents like COX-2 inhibitors, VEGF inhibitors, or other anti-angiogenic agents in metronomic therapy are still uncertain and their use as an adjuvant in metronomic regimens is still being studied. The same uncertainty also explains why not all anti-angiogenic agents can be expected to add a therapeutic effect in metronomic therapy [118]. The goal to enroll the best reagent in combination of metronomic therapy cannot be achieved unless more in-vitro and in-vivo studies provide more evidence about the safety profile, the risk of pharmacokinetic/dynamic interference, the ease of administration, and costs [117]. Third, it is difficult to define which population of cancer patients might be benefited from metronomic therapy and vice versa, and it is also difficult to determine which combination or schedule might be the best for patients with certain cancers, for example, gynecologic tumors. Finally, the best biomarker to use for tumor responsiveness or the prognosis of patients is still under evaluation. The prognostic relevance of TSP-1, bFGF, VEGF, VEGFR [119] CD31-microvessel density (MVD), VEGF-histoscore (HS), p53-HS, and TSP1 image analysis score (IA) [120] in CEO has been evaluated, and tumor-specific cell-free DNA levels have been assessed as an indicator of anti-tumor effect [51]. We need more accurate and reliable markers, like circulating endothelial cells or progenitor endothelial cells, to monitor the anti-angiogenic effects of these chronic schedules [117] to help physicians adjust the dosages and time intervals.

Agents used for targeted therapy against tumor cells are believed to be potent adjuvant agents in metronomic chemotherapy and deserve more thorough evaluation. With the advances in molecular and genetic techniques, relevant information on cancers will not only help us identify suitable patients but also design the proper target drugs [121]. By accumulating molecular-based knowledge of various and heterogeneous tumors, clinical trials can be held, negative results avoided, and more confidence gained [122]. Moreover, the development of *in vitro* and *in vivo* models to test these new therapeutic strategies will certainly help establish stronger preclinical evidence before clinical trials for these critical patients [121]. However, it is still difficult for us to study all the genomic components of the tumors and patients, so computerized simulation might be a solution to customize the best treatment regimen for individual patients suffering from a specific cancer.

Conclusion

This review addressed the mechanism of metronomic chemotherapy in the management of gynecologic cancers. Gynecologists have seen the successful application of a number of conventional cytotoxic drugs in this chronic administration schedule. The term "metronomic" refers mainly to the schedule, which consists of chronic, equally spaced, and generally low doses of single or combined chemotherapeutic drugs without extended drug-free breaks. The success of metronomic therapy was believed to rely on continuous administration, activation of cancer immunology, and antiangiogenic effects. Strategies devised for targeted adjuvant treatment to inhibit angiogenesis of malignant tumors include neutralization of circulating growth factors, and inhibition of RTK activation. Though there are still many unresolved questions regarding this new treatment regimen, it is definitely worthy of further research and evaluation, not only because of its potential to control advanced cancer, but also its fewer side effects and better life quality for cancer patients. The explosion in the number of new molecular targets and the development and application of many powerful technologies should accelerate the discovery of innovative molecular therapeutics, and eventually result in the identification of new therapeutic and prognostic targets [123].

Antiangiogenic "metronomic" scheduling of cytotoxic chemotherapeutics has shown favorable antitumor efficacy with limited toxicity and better results when combined with specific angiogenesis targeted agents. With joint efforts between basic and clinical research, a new paradigm carrying the prospect of turning cancer into a chronic and controllable disease can finally be achieved [124].

Key issues

1. Conventionally, cancer patients are usually treated at or near the maximum tolerated dose (MTD) with the implicit intent of eradicating (curing) the tumor after balancing between efficacy in tumor killing and toxicity to the host.
2. The concept that cancer could be considered as a chronic disease and might be treated like other chronic diseases to achieve a status called tumor dormancy is gaining popularity.
3. The practice, called metronomic therapy, has been increasing interest in putting more effort into administering cytotoxic drugs on a more continuous basis, with a much shorter break period, or none at all, and generally lower doses of various cytotoxic drugs or combinations with other newer, targeted therapies, like antiangiogenesis agents.
4. There are still many unresolved problems regarding the metronomic therapy for gynecologic cancers, including the optimal therapeutic dose of the antiangiogenic effect of the continuous use of cytotoxic agents is still unknown; the role of targeted agents like COX-2 inhibitors, VEGF inhibitors, or other antiangiogenic agents in metronomic therapy is still uncertain; and which population of cancer patients might be benefited from metronomic therapy and vice versa is difficult to determine; and the better biomarker to use for tumor responsiveness or the prognosis of patients is still under evaluation.

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References

[1] Wang PH, Chang C. Androgens and ovarian cancers. Eur J Gynaecol Oncol 2004;25:157−63.

[2] Wang PH, Lee WL, Juang CM, Yang YH, Lo WH, Lai CR, et al. Altered mRNA expressions of sialyltransferases in ovarian cancers. Gynecol Oncol 2005;99:63−9.

[3] Horng HC, Yuan CC, Lai CR, Wang PH. Presumed Stage IA primary epithelial ovarian carcinoma: the role of complete staging surgery. Eur J Gynaecol Oncol 2007;28:43−4.

[4] Gatenby RA, Silva AS, Gillies RJ, Frieden BR. Adaptive therapy. Cancer Res 2009;69:4894−903.

[5] McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 1996;334:1−6.

[6] Chen CH, Yang MJ, Cheng MH, Yen MS, Lai CR, Wang PH. Fertility preservation with treatment of immature teratoma of the ovary. J Chin Med Assoc 2007;70:218−21.

[7] Hanahan D, Bergers G, Bergsland E. Less is more regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest 2000;105:1045−7.

[8] Mutsaers AJ. Metronomic chemotherapy. Top Companion Anim Med 2009;24:137−43.

[9] Scharovsky OG, Mainetti LE, Rozados VR. Metronomic chemotherapy: changing the paradigm that more is better. Curr Oncol 2009;16:7−15.

[10] Kerbel RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004;4:423−36.

[11] Wang PH, Chao HT, Chao KC. Chemotherapy-induced gonadotoxicity. Taiwan J Obstet Gynecol 2010;49:1−2.

[12] Pan CC, Lee WL. Vaginal obliteration in a woman with a history of cutaneous T-cell lymphoma: the results of combined chemotherapy-induced gonadal toxicity and lymphoma relapse. Taiwan J Obstet Gynecol 2010;49:69−71.

[13] Gasparini G. Metronomic scheduling: the future of chemotherapy? Lancet Oncol 2001;2:733−40.

[14] Kamen BA, Rubin E, Aisner J, Glatstein E. High-time chemotherapy or high time for low dose. J Clin Oncol 2000;18:2935−7.

[15] Kerbel RS, Klement G, Pritchard KI, Kamen B. Continuous low-dose anti-angiogenic/ metronomic chemotherapy: from the research laboratory into the oncology clinic. Ann Oncol 2002;13:12−5.

[16] Skipper HE, Schabel Jr FM, Mellett LB, Montgomery JA, Wilkoff LJ, Lloyd HH, et al. Implications of biochemical cytokinetic pharmacologic and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 1970;54:431−50.

[17] Kamen BA, Glod J, Cole PD. Metronomic therapy from a pharmacologist's view. J Pediatr Hematol Oncol 2006;28:325−7.

[18] Nieto Y. The verdict is not in yet analysis of the randomized trials of high-dose chemotherapy for breast cancer. Haematologica 2003;88: 201−11.

[19] Roche H, Viens P, Biron P, Lotz JP, Asselain B. High-dose chemotherapy for breast cancer: the French PEGASE experience. Cancer Control 2003;10:42−7.

[20] Piccart-Gebhart MJ. Mathematics and oncology: a match for life? J Clin Oncol 2003;21:1425−8.

[21] Tuma RS. Dosing study seen as victory for clinical trials mathematical models. J Natl Cancer Inst 2003;95:254−5.

[22] Citron ML, Berry DA, Cirrincione C, Hudis C, Winer EP, Gradishar WJ, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol 2003;21:1431−9.

[23] Fidler IJ, Ellis LM. Chemotherapeutic drugs−more really is not better. Nat Med 2000;6:500−2.

[24] Kalyn R. Overview of targeted therapies in oncology. J Oncol Pharm Pract 2007;13:199−205.

[25] Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin DJ, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 2000;105:R15−24.

[26] Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS, et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 2000;60:1878−86.

[27] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182−6.

[28] Fioravanti A, Canu B, Ali G, Orlandi P, Allegrini G, Di Desidero T, et al. Metronomic 5-fluorouracil oxaliplatin and irinotecan in colorectal cancer. Eur J Pharmacol 2009;619:8−14.

[29 Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991−8.

[30] Kosmaczewska A, Ciszak L, Potoczek S, Frydecka I. The significance of Treg cells in defective tumor immunity. Arch Immunol Ther Exp Warsz 2008;56:181−91.

[31] Wong ML, Prawira A, Kaye AH, Hovens CM. Tumour angiogenesis: its mechanism and therapeutic implications in malignant gliomas. J Clin Neurosci 2009;16:1119−30.

[32] Burger RA. Experience with bevacizumab in the management of epithelial ovarian cancer. J Clin Oncol 2007;25:2902−8.

[33] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646−74.

[34] Cheng MH, Wang PH. Placentation abnormalities in the pathophysiology of preeclampsia. Expert Rev Mol Diagn 2009;9:37−49.

[35] Hanahan D. Signaling vascular morphogenesis and maintenance. Science 1997;277:48−50.

[36] Rehman S, Jayson GC. Molecular imaging of antiangiogenic agents. Oncologist 2005;10:92−103.

[37] Vacca A, Iurlaro M, Ribatti D, Minischetti M, Nico B, Ria R, et al. Antiangiogenesis is produced by nontoxic doses of vinblastine. Blood 1999;94:4143−55.

[38] Ng SS, Figg WD, Sparreboom A. Taxane-mediated antiangiogenesis in vitro: influence of formulation vehicles and binding proteins. Cancer Res 2004;64:821−4.

[39] Wang J, Lou P, Lesniewski R, Henkin J. Paclitaxel at ultra low concentrations inhibits angiogenesis without affecting cellular microtubule assembly. Anticancer Drugs 2003;14:13−9.

[40] Folkman J. Angiogenesis and apoptosis. Semin Cancer Biol 2003;13: 159−67.

[41] Bocci G, Nicolaou KC, Kerbel RS. Protracted low-dose effects on human endothelial cell proliferation and survival in vitro reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res 2002;2:6938−43.

[42] Pasquier E, Kavallaris M, Andre N. Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 2010;7:455−65.

[43] Hashimoto K, Man S, Xu P, Cruz-Munoz W, Tang T, Kumar R, et al. Potent preclinical impact of metronomic low-dose oral topotecan combined with the antiangiogenic drug pazopanib for the treatment of ovarian cancer. Mol Cancer Ther 2010;9:996−1006.

[44] Samaritani R, Corrado G, Vizza E, Sbiroli C. Cyclophosphamide "metronomic" chemotherapy for palliative treatment of a young patient with advanced epithelial ovarian cancer. BMC Cancer 2007;7:65.

[45] Kerbel RS. Improving conventional or low dose metronomic chemotherapy with targeted antiangiogenic drugs. Cancer Res Treat 2007;39:150−9.

[46] Sanchez-Munoz A, Mendiola C, Perez-Ruiz E, Rodriguez-Sanchez CA, Jurado JM, Alonso-Carrion L, et al. Bevacizumab plus low-dose metronomic oral cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. Oncology 2010;79:98−104.

[47] Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral

cyclophosphamide in recurrent ovarian cancer: a trial of the California Chicago and Princess Margaret Hospital phase II consortia. J Clin Oncol 2008;26:76–82.
[48] Jurado JM, Sanchez A, Pajares B, Perez E, Alonso L, Alba E. Combined oral cyclophosphamide and bevacizumab in heavily pretreated ovarian cancer. Clin Transl Oncol 2008;10:583–6.
[49] Merritt WM, Danes CG, Shahzad MM, Lin YG, Kamat A, Han LY, et al. Anti-angiogenic properties of metronomic topotecan in ovarian carcinoma. Cancer Biol Ther 2009;8:1596–603.
[50] Comander AH, Cannistra SA. A feasibility study of low-dose prolonged oral topotecan in patients with advanced ovarian fallopian tube or primary peritoneal serous cancer who have attained a complete clinical response following platinum-based chemotherapy. Int J Gynecol Cancer 2008;18:51–8.
[51] Kamat AA, Kim TJ, Landen Jr CN, Lu C, Han LY, Lin YG, et al. Metronomic chemotherapy enhances the efficacy of antivascular therapy in ovarian cancer. Cancer Res 2007;67:281–8.
[52] Albertsson P, Lennernas B, Norrby K. On metronomic chemotherapy: modulation of angiogenesis mediated by VEGE-A. Acta Oncol 2006; 45:144–55.
[53] Naganuma Y, Choijamts B, Shirota K, Nakajima K, Ogata S, Miyamoto S, et al. Metronomic doxifluridine chemotherapy combined with the anti-angiogenic agent TNP-470 inhibits the growth of human uterine carcinosarcoma xenografts. Cancer Sci 2011;102:1545–52.
[54] Choijamts B, Naganuma Y, Nakajima K, Kawarabayashi T, Miyamoto S, Tachibana K, et al. Metronomic irinotecan chemotherapy combined with ultrasound irradiation for a human uterine sarcoma xenograft. Cancer Sci 2011;102:452–9.
[55] Dushkin H, Schilder RJ. Imatinib mesylate and its potential implications for gynecologic cancers. Curr Treat Options Oncol 2005;6:115–20.
[56] Swift PS, Purser P, Roberts LW, Pickett B, Powell CB, Phillips TL. Pulsed low dose rate brachytherapy for pelvic malignancies. Int J Radiat Oncol Biol Phys 1997;37:811–7.
[57] Gupta AK, Vicini FA, Frazier AJ, Barth-Jones DC, Edmundson GK, Mele E, et al. A Iridium-192 transperineal interstitial brachytherapy for locally advanced or recurrent gynecological malignancies. Int J Radiat Oncol Biol Phys 1999;43:1055–60.
[58] Hall EJ, Brenner DJ. The dose-rate effect revisited: radiobiological considerations of importance in radiotherapy. Int J Radiat Oncol Biol Phys 1991;21:1403–14.
[59] Furre T, Koritzinsky M, Olsen DR, Pettersen EO. Inverse dose-rate effect due to pre-mitotic accumulation during continuous low dose-rate irradiation of cervix carcinoma cells. Int J Radiat Biol 1999;75: 699–707.
[60] Roberts WS, Lapolla JP, Greenberg H, Berman C, McDonald T. Continuous intra-arterial cisplatin combined with radiotherapy in locally advanced squamous carcinoma of the cervix: a Gynecologic Oncology Group pilot study. Int J Gynecol Cancer 1995;5:335–40.
[61] Micheletti E, La Face B, Bianchi E, Cagna E, Apostoli P, Ruggeri G, et al. Continuous infusion of carboplatin during conventional radiotherapy treatment in advanced squamous carcinoma of the cervix uteri IIB-IIIB UICC A phase I/II and pharmacokinetic study. Am J Clin Oncol 1997;20:613–20.
[62] Tonkin KS, Kelland LR, Steel GG. Chemotherapy-radiation interactions in human cervix carcinoma xenografts. Br J Cancer 1988;58:738–41.
[63] Kim JS, Kim SY, Kim KH, Cho MJ. Hyperfractionated radiotherapy with concurrent chemotherapy for para-aortic lymph node recurrence in carcinoma of the cervix. Int J Radiat Oncol Biol Phys 2003;55:1247–53.
[64] Marnitz S, Kohler C, Burova E, Wlodarczyk W, Jahn U, Grun A, et al. Helical tomotherapy with simultaneous integrated boost after laparoscopic staging in patients with cervical cancer: analysis of feasibility and early toxicity. Int J Radiat Oncol Biol Phys 2012;182:e137–43.
[65] Gruen A, Musik T, Kohler C, Fuller J, Wendt T, Stromberger C, et al. Adjuvant chemoradiation after laparoscopically assisted vaginal radical hysterectomy LARVH in patients with cervical cancer: Oncologic outcome and morbidity. Strahlenther Onkol 2011;187:344–9.
[66] Tewari KS, Monk BJ. Beyond platinum for metastatic and recurrent carcinoma of the cervix. Onkologie 2009;32:52–4.
[67] Zhang L, Zhang S. ZM447439 the Aurora kinase B inhibitor suppresses the growth of cervical cancer SiHa cells and enhances the chemosensitivity to cisplatin. J Obstet Gynaecol Res 2011;37:591–600.
[68] Hsu WL, Shueng PW, Jen YM, Wu CJ, Liu HS, Su CC, et al. Concurrent 5-fluorouracil daily low-dose cisplatin and radiotherapy in stage IIIB cervical cancer A phase II prospective study. Am J Clin Oncol 1996;19:263–7.
[69] Macchia G, Ferrandina G, Legge F, Deodato F, Ruggieri V, Lorusso D, et al. Prolonged chemoradiation in locally advanced carcinoma of the uterine cervix: final results of a phase II study ESTER-1. Am J Clin Oncol 2010;33:577–82.
[70] Kelland LR, Tonkin KS. The effect of 3-aminobenzamide in the radiation response of three human cervix carcinoma xenografts. Radiother Oncol 1989;15:363–9.
[71] Lin JC, Ho ES, Jan JS, Yang CH, Liu FS. High complete response rate of concomitant chemoradiotherapy for locally advanced squamous cell carcinoma of the uterine cervix. Gynecol Oncol 1996;61:101–8.
[72] Neijt JP. Advances in the chemotherapy of gynecologic cancer. Curr Opin Oncol 1994;6:531–8.
[73] Merritt WM, Nick AM, Carroll AR, Lu C, Matsuo K, Dumble M, et al. Bridging the gap between cytotoxic and biologic therapy with metronomic topotecan and pazopanib in ovarian cancer. Mol Cancer Ther 2010;9:985–95.
[74] Burger RA. Overview of anti-angiogenic agents in development for ovarian cancer. Gynecol Oncol 2011;121:230–8.
[75] Annunziata CM, Walker AJ, Minasian L, Yu M, Kotz H, Wood BJ, et al. Vandetanib designed to inhibit VEGFR2 and EGFR signaling had no clinical activity as monotherapy for recurrent ovarian cancer and no detectable modulation of VEGFR2. Clin Cancer Res 2010;16: 664–72.
[76] Palayekar MJ, Herzog TJ. The emerging role of epidermal growth factor receptor inhibitors in ovarian cancer. Int J Gynecol Cancer 2008;18: 879–90.
[77] Banerjee S, Gore M. The future of targeted therapies in ovarian cancer. Oncologist 2009;14:706–16.
[78] Ma WW, Jimeno A. Strategies for suppressing angiogenesis in gynecological cancers. Drugs Today Barc 2007;43:259–73.
[79] Grothey A, Ellis LM. Targeting angiogenesis driven by vascular endothelial growth factors using antibody-based therapies. Cancer J 2008;14: 170–7.
[80] Bando H. Vascular endothelial growth factor and bevacitumab in breast cancer. Breast Cancer 2007;14:163–73.
[81] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
[82] Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, et al. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc Natl Acad Sci U S A 2001;98:2604–9.
[83] Rak J. Is cancer stem cell a cell or a multicellular unit capable of inducing angiogenesis? Med Hypotheses 2006;66:601–4.
[84] Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 2007;6:273–86.
[85] Dizon DS. Where do antiangiogenic agents belong in the treatment algorithm for ovarian cancer? Am J Clin Oncol; 2011 May 5 [Epub ahead of print].
[86] Cannistra SA, Matulonis UA, Penson RT, Hambleton J, Dupont J, Mackey H, et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol 2007;25:5180–6.
[87] Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JI. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. J Clin Oncol 2007;25:5165–71.
[88] Chura JC, Van Iseghem K, Downs Jr LS, Carson LF, Judson PL. Bevacizumab plus cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. Gynecol Oncol 2007;107:326–30.
[89] Richardson DL, Backes FJ, Seamon LG, Zanagnolo V, O'Malley DM, Cohn DE, et al. Combination gemcitabine platinum and bevacizumab

for the treatment of recurrent ovarian cancer. Gynecol Oncol 2008;111: 461−6.
[90] Eleutherakis-Papaiakovou V, Bamias A, Dimopoulos MA. Thalidomide in cancer medicine. Ann Oncol 2004;15:1151−60.
[91] Gordinier ME, Dizon DS, Weitzen S, Disilvestro PA, Moore RG, Granai CO. Oral thalidomide as palliative chemotherapy in women with advanced ovarian cancer. J Palliat Med 2007;10:61−6.
[92] Hurteau JA, Brady MF, Darcy KM, McGuire WP, Edmonds P, Pearl ML, et al. Randomized phase III trial of tamoxifen versus thalidomide in women with biochemical-recurrent-only epithelial ovarian fallopian tube or primary peritoneal carcinoma after a complete response to first-line platinum/taxane chemotherapy with an evaluation of serum vascular endothelial growth factor VEGF: A Gynecologic Oncology Group Study. Gynecol Oncol 2010;119:444−50.
[93] Downs Jr LS, Judson PL, Argenta A, Ghebre R, Geller MA, Bliss RL, et al. A prospective randomized trial of thalidomide with topotecan compared with topotecan alone in women with recurrent epithelial ovarian carcinoma. Cancer 2008;112:331−9.
[94] Phippen NT, Leath CA. 3rd. Weekly topotecan and daily thalidomide in patients with recurrent epithelial ovarian cancer: a report of 2 cases. J Reprod Med 2009;54:583−6.
[95] Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, et al. ZD6474 inhibits vascular endothelial growth factor signaling angiogenesis and tumor growth following oral administration. Cancer Res 2002;62:4645−55.
[96] Ciardiello F, Caputo R, Damiano V, Troiani T, Vitagliano D, Carlomagno F, et al. Antitumor effects of ZD6474 a small molecule vascular endothelial growth factor receptor tyrosine kinase inhibitor with additional activity against epidermal growth factor receptor tyrosine kinase. Clin Cancer Res 2003;9:1546−56.
[97] Cesca M, Frapolli R, Berndt A, Scarlato V, Richter P, Kosmehl H, et al. The effects of vandetanib on paclitaxel tumor distribution and antitumor activity in a xenograft model of human ovarian carcinoma. Neoplasia 2009;11:1155−64.
[98] Isenberg JS, Martin-Manso G, Maxhimer JB, Roberts DD. Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. Nat Rev Cancer 2009;9:182−94.
[99] Greenaway J, Henkin J, Lawler J, Moorehead R, Petrik J. ABT-510 induces tumor cell apoptosis and inhibits ovarian tumor growth in an orthotopic syngeneic model of epithelial ovarian cancer. Mol Cancer Ther 2009;8:64−74.
[100] Campbell NE, Greenaway J, Henkin J, Moorehead RA, Petrik J. The thrombospondin-1 mimetic ABT-510 increases the uptake and effectiveness of cisplatin and paclitaxel in a mouse model of epithelial ovarian cancer. Neoplasia 2010;12:275−83.
[101] Barnes MN, Chhieng DF, Dreher M, Jones JL, Grizzle WE, Jones L, et al. Feasibility of performing chemoprevention trials in women at elevated risk of ovarian carcinoma: initial examination of celecoxib as a chemopreventive agent. Gynecol Oncol 2005;98:376−82.
[102] Liu H, Xiao J, Yang Y, Liu Y, Ma R, Li Y, et al. COX-2 expression is correlated with VEGF-C lymphangiogenesis and lymph node metastasis in human cervical cancer. Microvasc Res 2011;82:131−40.
[103] Legge F, Paglia A, D'Asta M, Fuoco G, Scambia G, Ferrandina G. Phase II study of the combination carboplatin plus celecoxib in heavily pre-treated recurrent ovarian cancer patients. BMC Cancer 2011;11: 214.
[104] Vital-Reyes V, Rodriguez-Burford C, Chhieng DC, Oelschlager DK, Reyes-Fuentes A, Barnes M, et al. Celecoxib inhibits cellular growth decreases Ki-67 expression and modifies apoptosis in ovarian cancer cell lines. Arch Med Res 2006;37:689−95.
[105] Bijman MN, Hermelink CA, van Berkel MP, Laan AC, Janmaat ML, Peters GJ, et al. Interaction between celecoxib and docetaxel or cisplatin in human cell lines of ovarian cancer and colon cancer is independent of COX-2 expression levels. Biochem Pharmacol 2008;75:427−37.
[106] Geisinger KR, Berens ME, Duckett Y, Morgan TM, Kute TE, Welander CE. The effects of estrogen progesterone and tamoxifen alone and in combination with cytotoxic agents against human ovarian carcinoma in vitro. Cancer 1990;65:1055−61.
[107] Hasan J, Ton N, Mullamitha S, Clamp A, McNeilly A, Marshall E. Jayson G C Phase II trial of tamoxifen and goserelin in recurrent epithelial ovarian cancer. Br J Cancer 2005;93:647−51.
[108] Kanwar VS, Heath J, Krasner CN, Pearce JM. Advanced small cell carcinoma of the ovary in a seventeen-year-old female successfully treated with surgery and multi-agent chemotherapy. Pediatr Blood Cancer 2008;50:1060−2.
[109] McMeekin DS, Sill MW, Darcy KM, Stearns-Kurosawa DJ, Webster K, Waggoner S, et al. phase II trial of thalidomide in patients with refractory leiomyosarcoma of the uterus and correlation with biomarkers of angiogenesis: a gynecologic oncology group study. Gynecol Oncol 2007;106:596−603.
[110] Ren W, Korchin B, Lahat G, Wei C, Bolshakov S, Nguyen T, et al. Combined vascular endothelial growth factor receptor/epidermal growth factor receptor blockade with chemotherapy for treatment of local uterine and metastatic soft tissue sarcoma. Clin Cancer Res 2008; 14:5466−75.
[111] Wright JD, Powell MA, Rader JS, Mutch DG, Gibb RK. Bevacizumab therapy in patients with recurrent uterine neoplasms. Anticancer Res 2007;27:3525−8.
[112] Gaffney DK, Winter K, Dicker AP, Miller B, Eifel PJ, Ryu J, et al. Efficacy and patterns of failure for locally advanced cancer of the cervix treated with celebrex celecoxib and chemoradiotherapy in RTOG 0128. Int J Radiat Oncol Biol Phys 2007;69:111−7.
[113] Gaffney DK, Winter K, Dicker AP, Miller B, Eifel PJ, Ryu J, et al. A Phase II study of acute toxicity for Celebrex celecoxib and chemoradiation in patients with locally advanced cervical cancer: primary endpoint analysis of RTOG 0128. Int J Radiat Oncol Biol Phys 2007;67: 104−9.
[114] Herrera FG, Chan P, Doll C, Milosevic M, Oza A, Syed A, et al. A prospective phase I-II trial of the cyclooxygenase-2 inhibitor celecoxib in patients with carcinoma of the cervix with biomarker assessment of the tumor microenvironment. Int J Radiat Oncol Biol Phys 2007;67: 97−103.
[115] Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. J Clin Oncol 2009;27:1069−74.
[116] Monk BJ, Willmott LJ, Sumner DA. Anti-angiogenesis agents in metastatic or recurrent cervical cancer. Gynecol Oncol 2010;116:181−6.
[117] Emmenegger U, Kerbel RS. Five years of clinical experience with metronomic chemotherapy: achievements and perspectives. Onkologie 2007;30:606−8.
[118] Colleoni M, Orlando L, Sanna G, Rocca A, Maisonneuve P, Peruzzotti G, et al. Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: antitumor activity and biological effects. Ann Oncol 2006;17:232−8.
[119] Secord AA, Darcy KM, Hutson A, Lee PS, Havrilesky LJ, Grace LA, et al. Co-expression of angiogenic markers and associations with prognosis in advanced epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol 2007;106:221−32.
[120] Han ES, Burger RA, Darcy KM, Sill MW, Randall LM, Chase D, et al. Predictive and prognostic angiogenic markers in a gynecologic oncology group phase II trial of bevacizumab in recurrent and persistent ovarian or peritoneal cancer. Gynecol Oncol 2010;119(3):484−90.
[121] Idbaih A, Ducray F, Sierra Del Rio M, Hoang-Xuan K, Delattre JY. Therapeutic application of noncytotoxic molecular targeted therapy in gliomas: growth factor receptors and angiogenesis inhibitors. Oncologist 2008;13:978−92.
[122] Kummar S, Kinders R, Rubinstein L, Parchment RE, Murgo AJ, Collins J, et al. Compressing drug development timelines in oncology using phase '0' trials. Nat Rev Cancer 2007;7:131−9.
[123] Kikuchi Y, Kita T, Takano M, Kudoh K, Yamamoto K. Treatment options in the management of ovarian cancer. Expert Opin Pharmacother 2005;6:743−54.
[124] Gille J, Spieth K, Kaufmann R. Metronomic low-dose chemotherapy as antiangiogenic therapeutic strategy for cancer. J Dtsch Dermatol Ges 2005;3:26−32.