THE ANTI-ANGIOGENIC BASIS OF METRONOMIC CHEMOTHERAPY

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In addition to proliferating cancer cells and various types of normal cells, such as those of the bone marrow, conventional cytotoxic chemotherapeutics affect the endothelium of the growing tumour vasculature. The anti-angiogenic efficacy of chemotherapy seems to be optimized by administering comparatively low doses of drug on a frequent or continuous schedule, with no extended interruptions — sometimes referred to as 'metronomic' chemotherapy. In addition to reduced acute toxicity, the efficacy of metronomic chemotherapy seems to increase when administered in combination with specific anti-angiogenic drugs. Gaining better insight into the mechanisms of these effects could lessen or even eliminate the empiricism used to determine the optimal dose and schedule for metronomic chemotherapy regimens.

For almost half a century, systemic therapy of cancer has been dominated by the use of cytotoxic chemotherapeutics. Most of these drugs are DNA-damaging agents or microtubule inhibitors that are designed to inhibit or kill rapidly dividing cells. They are often administered in single doses or short courses of therapy at the highest doses possible without causing life-threatening levels of toxicity — this is referred to as the 'maximum tolerated dose' (MTD). MTD therapy requires prolonged breaks (generally of 2–3 weeks in duration) between successive cycles of therapy. Despite the number of such chemotherapeutics and the huge number of clinical trials that have been undertaken to test them, progress has been modest in terms of curing or significantly prolonging the lives of patients with cancer — particularly those with advanced-stage or metastatic disease[1,2]. Moreover, the progress that has been made in treating certain types of malignancy often comes at a high price, given the toxic side effects that are frequently associated with MTD-based chemotherapy. These include acute myelosuppression, hair loss, damage to the intestinal mucosa, nausea and mucositis, as well as the long-term cardiac, renal, neurological and reproductive consequences. Indeed, many of the recent pharmacological advances in oncology treatment involve growth factors and anti-nausea drugs, which are administered to patients with cancer to minimize the severity of, or accelerate recovery from, chemotherapy-induced toxicities. Such 'supportive-care drugs' can significantly add to the financial burden of cancer chemotherapy, and have their own side effects.

A reappraisal of the best ways of administering chemotherapy is underway. Instead of only using short bursts of toxic MTD chemotherapy interspersed with long breaks to allow recovery from the harmful side effects, 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 — not only in terms of reducing certain toxicities, but perhaps even improving antitumour effects as well[3–6]. Moreover, some of these dosing/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. The most recent refinement of this concept is called 'metronomic' chemotherapy[3], which refers to the frequent, even daily, administration of chemotherapeutics at doses significantly below the MTD, with no prolonged drug-free breaks.

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Summary

- Conventional cytotoxic anticancer drugs have anti-angiogenic effects, which could contribute to their antitumour efficacy.
- The anti-angiogenic effects of chemotherapy seem to be optimized by administering such drugs 'metronomically' — in small doses on a frequent schedule (daily, several times a week, or weekly) in an uninterrupted manner, for prolonged periods.
- Conventional chemotherapy, which is administered at more toxic 'maximum tolerated doses', requires 2–3-week breaks between successive cycles of therapy. This seems to counteract the potential for sustained, therapeutically effective anti-angiogenic effects.
- In preclinical models, metronomic chemotherapy can be effective in treating tumours in which the cancer cells have developed resistance to the same chemotherapeutics. This also has the advantage of being less acutely toxic, therefore making more prolonged treatments possible.
- The efficacy of metronomic chemotherapy can be significantly increased when administered in combination with anti-angiogenic drugs, such as antibodies against vascular endothelial growth factor (VEGF) or VEGF receptor 2.
- Some metronomic-chemotherapy regimens induce sustained suppression of circulating endothelial progenitor cells and increase the levels of the endogenous angiogenesis inhibitor thrombospondin 1, both of which can suppress neovascularization.
- Clinical trials are under way to test several combinations of metronomic chemotherapy and anti-angiogenic drugs.

Metronomic therapy

There are many different factors that have contributed to the line of reasoning that for chemotherapy, 'the more frequent the better' and that 'less is more'. First, the opposite approach — using 'high-dose' chemotherapy with autologous bone-marrow stem-cell transplants (to replace the destroyed bone-marrow-derived stem cells) — has not provided the kind of survival benefits expected, at least when this treatment strategy is used for patients with metastatic breast cancer[7,8]. This approach is also very expensive and highly toxic. Furthermore, 'dose-dense' chemotherapy, in which one or more chemotherapeutic is administered at more frequent intervals (that is, every other week), has shown clear benefits in randomized Phase III clinical trials[9–11]. This strategy is usually designed to administer at least the same amount or, more commonly, even a greater amount of drug in total over time.[9]

So, if every other week is better than every 3 weeks, then why not administer weekly or even daily treatment? Indeed, it is becoming more common to administer taxane drugs to patients with certain types of cancer, such as breast cancer, on a weekly schedule[12–15]. Such dose density — which is allowable because of exogenously administered supportive-care growth factors, antibiotics and transfusion medicine — seems in some respects to be conforming to the metronomic-therapy theme, as discussed below.

METRONOMIC CHEMOTHERAPY can be viewed as a variation of dose-dense therapy with the exception that the cumulative dose with metronomic therapy might be significantly less than with MTD-based chemotherapy[15–16]. As metronomic therapy reduces the level of toxicity, it lessens or even removes the need for growth-factor support to accelerate recovery from myelosuppression. Moreover, despite lower cumulative doses of drug administration, the antitumour effects of this approach, in terms of prolonging survival times, might actually be superior to conventional MTD regimens, especially in some preclinical models[17–19]. Support for metronomic therapy also comes from mathematical modelling studies[20,21]. Unlike dose-dense chemotherapy, the main targets of which are presumed to be proliferating tumour cells, the main targets of frequent or continuous metronomic chemotherapy are the endothelial cells of the growing vasculature of a tumour[22]. In essence, chemotherapeutics are used as anti-angiogenic agents, therefore "redefining the target of chemotherapy", to cite Miller *et al.*[23]. This is also the reason that Browder *et al.*[22] coined the term 'anti-angiogenic chemotherapy' to describe this treatment strategy.

Another advantage of metronomic chemotherapy is the possibility of combining it with anti-angiogenic drugs, as well as other types of targeted therapies — such those that target specific signal-transduction molecules — or with antitumour vaccines. It is ironic that targeted therapies were originally designed with the goal of replacing chemotherapy, to reduce the serious morbidities associated with standard MTD or high-dose chemotherapy. However, although they are less toxic, most of these rationally designed drugs were found to have very modest efficacy, at least when used as single agents in treating patients with advanced disease. They have therefore mainly been used in combination with standard chemotherapy or radiation protocols. An example of this is bevacizumab (Avastin) — a humanized monoclonal antibody against vascular endothelial cell growth factor (VEGF) — which is used in combination with 5-fluorouracil (5-FU)/leucovorin/irinotecan for the treatment of metastatic colorectal cancer[24,25]. Another example is trastazumab (Herceptin) — a humanized monoclonal antibody against the ERBB2 oncoprotein — which is combined with an alkylating agent or paclitaxel for the treatment of metastatic breast cancer[26].

One of the proposed benefits of targeted therapies was reduced toxicity and improved quality of life. When these drugs are combined with MTDs of chemotherapy, however, these benefits are not realized. As it is likely that chemotherapy will continue to be the mainstay for systemic cancer therapy for many years to come, designing more effective ways of administering and combining such drugs with the newest generation of molecularly targeted drugs will become increasingly crucial.

Anti-angiogenic effects

Chemotherapeutics do not specifically target tumour cells, but rather interfere with cell division, such as by inhibiting enzymes involved DNA replication or metabolism (for example, topoisomerases and thymidylate synthase), or microtubules. These drugs therefore also damage the normal dividing cells of rapidly regenerating tissues, such as those of the bone marrow and gut mucosa, and hair-follicle cells. Host toxicity is therefore often only marginally less than antitumour efficacy, so creating a narrow therapeutic index.

METRONOMIC CHEMOTHERAPY
Chronic administration of chemotherapy at relatively low, non-toxic doses on a frequent schedule of administration, with no prolonged drug-free breaks.

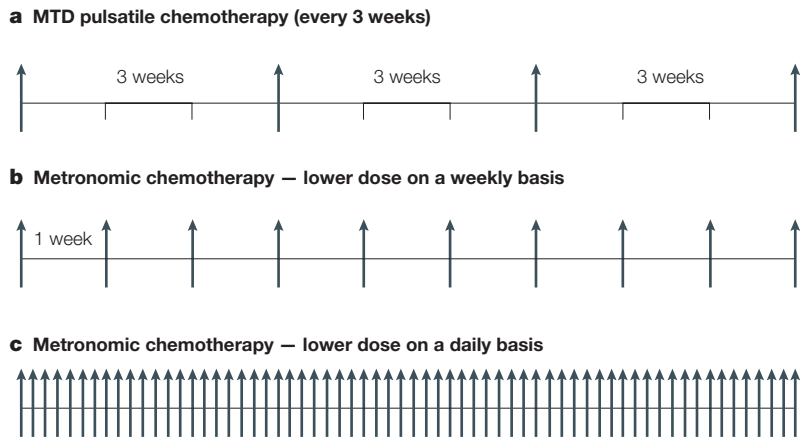

Figure 1 | **Different therapeutic regimens.** Metronomic chemotherapy regimens differ from the standard maximum tolerated dose (MTD) chemotherapy regimens that have been common practice in medical oncology for decades. **a** | In standard chemotherapy, a drug is typically given in a single bolus injection or infusion at the MTD, interspersed by a long break — for example, 3 weeks — before the next course of this therapy is administered. Doses that exceed the MTD ('high-dose' chemotherapy) must be accompanied by an autologous bone-marrow stem-cell transplant and supportive-care growth-factor drugs to prevent lethal bone-marrow failure. In **b** and **c**, examples of metronomic chemotherapy regimens are shown where, for example, the chemotherapy drug is administered more frequently, such as weekly (**b**) or daily (**c**), with no prolonged drug-free interruptions. Drugs that can be administered orally, such as cyclophosphamide, capecitabine, etoposide (VP-16), UFT (uracil plus tegafur, a fluoropyrimidine antimetabolite), would be ideal for prolonged daily administration schedules. Omission of prolonged drug-free periods is a key aspect of the basis for the anti-angiogenic effects of low-dose metronomic chemotherapy regimens, as these breaks allow repair and recovery from the anti-angiogenic effects of chemotherapy drugs on developing tumour blood vessels.

But perhaps there is a silver lining in this otherwise dark cloud, in that dividing endothelial cells are present in the growing blood vessels that are found in tumours[27] and, like other normal dividing cells, should be susceptible to chemotherapeutics[28]. Elimination of these dividing endothelial cells, or inhibition of their division, would presumably lead to an anti-angiogenic effect. Moreover, as host vascular endothelial cells are assumed to be genetically stable and lack the diverse genetic defects characteristic of cancer cells that lead to drug resistance, the putative effects of chemotherapy might be more durable in the face of continued therapy. By way of example, successive cycles of MTD-based chemotherapy can cause myelosuppression each time, the extent of which does not change appreciably[28]. If normal bone-marrow-cell progenitors acquired resistance to chemotherapy in the same way that genetically unstable, highly mutable cancer cells do, myelosuppression would gradually decline and disappear. So, the cancer cells that are resistant to a particular chemotherapeutic agent might indirectly respond to that same drug through a 'side effect' — loss of or damage to its associated vasculature, as first proposed in 1991 (REF. 29). Literature dating back to the mid-1980s shows that virtually every class of chemotherapeutic has anti-angiogenic effects or antivascular effects in various *in vitro* and *in vivo* assays[23].

Many tumours, however, are intrinsically drug resistant or rapidly acquire resistance after showing initial responsiveness to chemotherapy regimens. So it would seem that chemotherapy has minimal or negligible anti-angiogenic effects. Why is this? Perhaps the proportion of dividing endothelial cells in tumour-associated blood vessels is simply too low for chemotherapy to have a significant therapeutic impact. Alternatively, the endothelial cells might be protected from chemotherapy-induced cell death by high local concentrations of endothelial-cell survival factors such as VEGF, basic fibroblast growth factor (bFGF) and angiopoietin 1 (REFS 30,31). A third explanation, uncovered in a pioneering study from Judah Folkman's laboratory[22], is that the anti-angiogenic effects of chemotherapy are both masked and marginalized by the way chemotherapy is usually administered. In this case, the long breaks between drug administration that are necessary to allow the patient to recover from the harmful side effects of the MTD chemotherapy, especially from myelosuppression, reduce the anti-angiogenic effects of the drugs.

Timothy Browder and colleagues evaluated the anti-angiogenic and antitumour effects of the alkylating agent cyclophosphamide in immune-competent syngeneic mice that had been injected subcutaneously with various tumour types[22]. They found that this drug, when administered at the MTD, caused apoptosis of endothelial cells in the newly formed tumour microvessels[22]. A detailed temporal analysis showed that the endothelial cells were the first in the tumour to undergo apoptosis[22]. This anti-angiogenic effect did not, however, translate into a significant therapeutic benefit, apparently because the damage to the vasculature of the tumour was largely repaired during the long (2–3-week) rest/recovery periods between successive cycles of MTD-based therapy.

It was therefore proposed that if cyclophosphamide was given more frequently (FIG. 1), such as once or more per week with no extended breaks, there would be significantly less opportunity for repair of the damaged endothelium and the anti-angiogenic effects of the chemotherapy would irreversibly accumulate. This, of course, necessitates lowering the dose of the drug administered with each injection. Browder *et al.* showed that this more frequent, regular, lower-dose therapy, which was administered at one-third of the MTD, had impressive anti-angiogenic and antitumour effects when tested on several mouse tumour cell lines grown subcutaneously in syngeneic mice[22]. This approach allowed even very large established subcutaneous tumours, previously selected *in vivo* for acquired cyclophosphamide resistance using a conventional MTD regimen, to respond to the same drug and almost completely regress. In short, a state of acquired drug resistance could be reversed simply by apparently shifting the focus of the treatment away from the drug-resistant cancer-cell population to the drug-sensitive tumour endothelium[3,22].

These results have been confirmed by others[32,33] and have also been modified with daily oral administration of the drug through drinking water, which seems to be less toxic than the weekly regimen[19,34]. Indeed, a recent detailed analysis showed that long-term daily low-dose cyclophosphamide therapy did not cause significant

toxicity to tissues or cells normally affected by MTD regimens of the same drug[35]; lymphopaenia was the only toxic side effect noted[35].

Clinical precedents for metronomic therapy

In retrospect, these preclinical results actually have many intriguing clinical precedents[4,36]. For example, 40% of patients with non-small-cell lung cancer (NSCLC) who showed no response to standard doses of intravenous etoposide administered intermittently did respond — that is, their tumours shrank by 50% of more in volume — to the same drug when it was given orally at a much lower dose using a much more frequent basis (every day or every other day), with only a 1-week break every month[37]. Similar results have been shown in patients who have been given other drugs, such as microtubule-inhibiting taxanes, for treatment of advanced metastatic breast or ovarian cancer. In these patients, weekly regimens of drug administration are being increasingly adopted, often using only 30–40% of the MTD given once every 3 weeks[38]. In women who had stopped responding to the MTD of paclitaxel or docetaxel given once every 3 weeks, tumours were found to respond in a high proportion of cases to a regimen of approximately 30–40% the MTD once every week[13,36,38–40]. However, for the most part, these are not standard-of-care regimens and their benefits remain to be validated in randomized prospective Phase III clinical trials.

Metronomic therapy is also similar in many ways to the various long-term 'maintenance' chemotherapy regimens[41–45] that are used to treat children with certain types of cancer, such as acute lymphoblastic leukaemia. Maintenance therapy in this case involves the administration of low doses of oral methotrexate on a weekly basis and 6-mercaptopurine on a daily basis for up to 3 years. This treatment follows short-term remission-inducing chemotherapy using standard regimens and higher doses of various chemotherapeutic drugs[42,45]. Several studies have indicated that the drugs used in this type of maintenance therapy have anti-angiogenic effects[46–48]. In the case of methotrexate, low doses have been shown to cause anti-endothelial/anti-angiogenic effects in *in vitro* and *in vivo* assays[46,47]. Furthermore, the maintenance therapy used to treat patients with acute lymphoblastic leukaemia has been shown to have anti-angiogenic effects in the bone marrow, reducing the number of blood vessels in this tissue compartment[49–51]. The success of following standard MTD 'remission-induction' chemotherapy with long-term metronomic therapy regimens highlights the possibility that these two types of dosing regimens are not mutually exclusive, but can be used sequentially in a beneficial and harmonious manner. This approach should also be considered for adults, especially when combined with a cytostatic agent for long-term therapy.

Combination with anti-angiogenic drugs

Clinical trials are underway to determine whether metronomic chemotherapy can prolong survival when compared with standard MTD regimens in patients with various cancers, including advanced prostate and ovarian carcinomas, as well as certain types of haematological malignancies[6,49–52]. Relapses, however, will undoubtedly occur in most patients who initially show some benefit from metronomic therapy[49]. It was partly for this reason that the metronomic-chemotherapy protocol of Browder *et al.* has been modified and combined with endothelial-cell-specific angiogenesis inhibitors such as anti-VEGF receptor 2 (VEGFR2, also known as KDR or FLK1) antibodies or TNP-470, a fumigillin analogue[22,53]. This combination approach might improve efficacy without significantly increasing host toxicity[16].

The rationale for this strategy was based on several considerations. VEGF-receptor tyrosine kinases are expressed preferentially by endothelial cells of the growing neovasculature of a tumour, and VEGF is a key survival (anti-apoptotic) factor for the endothelial cells of newly formed vessels[54,55]. There are several signalling pathways and molecular effector mechanisms by which VEGF can inhibit apoptosis in endothelial cells[31,56–60]. For example, signalling through VEGFR2 can activate the phosphatidylinositol 3-kinase (PI3K)–AKT pro-survival signalling pathway. This or other pathways lead to subsequent upregulation of several anti-apoptotic effectors, including BCL2, A1, XIAP and survivin[31].

There is evidence that the anti-proliferative[30] or pro-apoptotic actions of pacitaxel[31], vinblastine, cisplatinum and adriamycin[31], as well as of several other types of cytotoxic substances, on human endothelial cells in culture are suppressed by the presence of VEGF[30,31]. High local concentrations of VEGF in the tumour microenvironment might therefore induce or promote multidrug resistance, by inducing a highly specific chemoprotective effect towards the VEGFR2-positive endothelial cells of the tumour[30,31,61]. Chemotherapy itself might also induce or upregulate the expression of VEGF and other endothelial-cell pro-survival growth factors in tumour cells[62]. So, the combination of a chemotherapeutic agent with a drug that blocks VEGF or its receptor (VEGFR2) should selectively amplify the pro-apoptotic effects of the chemotherapeutic against activated endothelial cells, but presumably not against other types of dividing normal cells[16]. This would improve the therapeutic index.

Previous studies have shown that anti-angiogenic drugs can improve the effects of some standard chemotherapy regimens[63,64], and these findings have been validated in the clinic[24,25]. For example, in a large, randomized, placebo-controlled Phase III clinical trial, the combination of a standard, approved chemotherapy regimen for metastatic colorectal cancer — consisting of 5-FU/leucovorin and irinotecan — with bevacizumab (the anti-VEGF antibody), caused a statistically significant prolongation of survival, compared with patients treated with only the chemotherapy regimen[25]. So, one might anticipate that anti-angiogenic drugs should also improve the efficacy of continuous low-dose chemotherapy regimens, for which the side effects would be much more tolerable, and that the two types of drug could be administered together for long time periods.

Experiments were undertaken to evaluate this combination treatment concept using xenograft models of neuroblastoma[16], melanoma, breast, prostate and colon cancers[19,65]. In one of these studies, a very low dose of vinblastine was administered twice weekly — which was about 1/10–1/20 the MTD for mice and therefore represents low-dose chemotherapy[66,67] — in combination with an anti-VEGFR2 blocking monoclonal antibody called DC101 (REF. 70). This combination caused complete and sustained regression of large, established — but localized — neuroblastoma xenografts in severe combined immunodeficient (SCID) mice. The metronomic vinblastine treatment was preceded by a 3-week remission-induction schedule of higher cumulative doses of the drug[16] to reduce the large tumour burden. This combination treatment, in which the two different drugs were given twice a week with no breaks, could be maintained for these exceptionally long periods (7 months) because they were not toxic to the mice[16]. By contrast, treatment with either the vinblastine regimen or DC101 alone, although non-toxic, resulted in significant but short delays in the growth of the tumour in the growth followed by relapse, and the animals only survived 1–2 months after initiation of treatment.

Were the low-dose chemotherapy regimens used in these studies anti-angiogenic and, if so, was this was the only antitumour mechanism? To address this question, a quantitative *in vivo* blood-vessel PERFUSION ASSAY showed that the low-dose vinblastine protocol itself could significantly inhibit angiogenesis[16]. Similarly, Browder *et al.* tested the weekly low-dose cyclophosphamide regimen using a CORNEAL MICROPOCKET ASSAY to show that this metronomic therapy protocol inhibited angiogenesis[22].

Other studies involved[65] human breast cancer cell lines that had been previously selected for resistance to agents such as paclitaxel, doxorubicin and vinblastine, as a result of overexpression of the multidrug resistance 1 (*MDR1*) gene, which encodes the P-glycoprotein drug-efflux pump[65]. In some cases, these cells were resistant to 50–100-fold higher concentrations of drugs than normal cells[65]. In xenograft studies, the combination of DC101 plus metronomic chemotherapy slowed tumour growth, whereas DC101 treatment or metronomic chemotherapy alone resulted in only temporary tumour responses — or even no apparent primary tumour responses, as measured by decreases in tumour volume. Cancer-cell-specific effects of drugs are not sufficient to overcome this level of drug resistance, so some alternative mechanism, such as anti-angiogenesis, must be involved[65]. Furthermore, Browder *et al.* administered a combination of the angiogenesis inhibitor TNP470 with weekly doses of cyclophosphamide to treat large, established transplanted mouse tumours that were previously selected for resistance to cyclophosphamide. They found that the combined treatment could gradually cause marked and sustained regressions, if not complete disappearance of such tumours[22].

The results of Browder *et al.* and Klement *et al.* have now been confirmed by many different groups using various empirical continuous or frequent low-dose chemotherapy regimens. These regimens included many different chemotherapeutic drugs, as well as several different anti-angiogenic agents[32,68–73]. A summary of the drugs, drug combinations and tumour models studied is shown in TABLE 1 (REFS 18,19,73,74). In some of these studies, an empirical metronomic dosing schedule was compared head-to-head with the respective MTD regimen of the same chemotherapeutic drug[17,19,20,71,72]. In all cases, the MTD regimens were found to be inferior to the metronomic treatments in terms of either toxicity or survival, or both. This also held for situations in which an anti-angiogenic drug was added to the MTD, compared with respective metronomic chemotherapy regimens[17,19,20].

The number of such studies, however, is limited and these findings need to be confirmed using different chemotherapeutic agents. Moreover, as discussed above, there might be additional benefits to using standard MTD chemotherapy followed by a subsequent long-term metronomic regimen — especially when treating exceptionally large tumours that are known to be responsive to certain chemotherapy drugs administered in the MTD fashion. This approach was used in preclinical studies by Klement *et al.*[16] and Bocci *et al.*[34]. Whereas some of the preclinical studies reported exceptionally long-term tumour responses, and in some cases mice were even cured[16,22], most mice eventually relapsed[75,76]. This indicates that some forms of acquired resistance occur — either at the host level (such as through altered drug metabolism), the tumour-cell level, (such as through selection for mutant tumour cells that can survive under the hypoxic conditions created by inhibition of angiogenesis)[75] or at the level of the endothelial cells or blood vessels (such as vascular remodelling into more mature vessels that are less responsive to anti-angiogenic treatment)[76].

Anti-angiogenic mechanisms

Much evidence, mostly *in vitro*, indicates that the 'activated' endothelial cells of newly forming blood-vessel capillaries are highly and selectively sensitive to very low doses of various chemotherapeutic drugs[47,77–81]. For example, several studies have been undertaken to test the antiproliferative, migration-inhibitory and sometimes cytotoxic effects of picomolar concentrations of chemotherapeutic drugs on various human cell types, including fibroblasts, lymphocytes, tumour cells, epithelial cells from various tissues, and microvascular or macrovascular endothelial cells. A summary of some of these studies is shown in TABLE 2. Some of the most interesting studies involve various microtubule inhibitors, such as vinblastine, paclitaxel and docetaxel. In these experiments, ultra-low concentrations of these drugs were reported to inhibit proliferation or migration of endothelial cells, but not of other cell types examined. For example, Wang *et al.* reported that 10–100,000-fold higher concentrations of paclitaxel were required to inhibit proliferation and migration of human astrocytes, fibroblasts, mammary epithelial cells, keratinocytes, prostate epithelial cells or smooth-muscle cells, compared with epithelial cells[80]. Taxanes, however, must be formulated in certain vehicles for injection, to

MATRIGEL-PERFUSION ASSAY OF ANGIOGENESIS
An assay that is widely used to measure angiogenesis. In this assay, an extracellular matrix gel-like plug (Matrigel) that contains angiogenic factors is implanted into the skin of mice. The new blood vessels that grow into the plug can be quantified by measuring perfusion of haemoglobin or large fluorescently tagged molecules (such as intravenously admininstered dextran) into the plugs.

CORNEAL-MICROPOCKET ANGIOGENESIS ASSAY
An assay for angiogenesis in which an inert polymer that contains an angiogenic growth factor, such as bFGF or VEGF, is implanted into the avascular cornea of mice or rabbits. This induces new blood vessels that can be visualized and quantified.

Table 1 | **Preclinical examples of antitumour efficacy of metronomic chemotherapy**

Tumour model	Drug combination tested	Results/comments	References
Large, established mouse tumours (EMT/6, Lewis Lung carcinoma or L1210 leukaemia)	Weekly CTX (150 mg/kg) with and without TNP-470	Regression of large drug-sensitive or resistant tumours; addition of TNP-470 required to regress drug-resistant tumours	22
Large, established s.c. (ectopic) human neuroblastoma xenografts in SCID mice	Twice per week very low-dose vinblastine (0.33 mg/kg or 1 mg/m^2) plus twice per week anti-VEGFR2 antibody (DC101) after upfront remission-induction vinblastine regimen	Sustained tumour regression with no relapse or toxicity	16
Established orthotopic multidrug resistant (P-gp-positive) human breast cancer xenografts in SCID mice	Twice per week low-dose paclitaxel or 2 times per week vinblastine, or 3 times per week cisplatinum adriamycin, with DC101 or anti-VEGFR2 antibody	Stabilization of tumours and prolonged survival in mice treated with combination therapy	65
Orthotopic human breast cancer (MDA-MB-231) xenografts in SCID mice	Daily, oral low-dose CTX (for example, ~20–40 mg/kg) plus DC101 anti-VEGFR2 antibody	Tumour stabilization or delayed growth, and prolonged survival with little toxicity	19
Advanced (late stage), bulky and spontaneous islet-cell pancreatic carcinomas arising in a mouse model of pancreatic cancer	Daily oral CTX (10 mg/kg) or twice per week vinblastine (1.5 mg/m^2) with either daily SU5416 VEGFR2 inhibitor or daily oral BA-1-12-9566 MMP inhibitor or daily i.p. BB-94 MMP inhibitor	Prolongation of survival and/or tumour regression (or stabilization), especially noted with low-dose CTX and BB-94 combination; drugs are generally ineffective as single agents	142
Orthotopic, established human U87 gliomablastoma xenograft	Frequent low-dose carboplatin and etoposide plus PEX fragment of MMP2, compared to standard chemotherapy plus PEX	Greatest survival benefit observed in low-dose chemotherapy plus PEX, and no side effects detected; severe side effects detected in higher (standard) dose chemotherapy group	18
Orthotopic human Wilms' tumour xenograft with lung metastases	Topotecan 0.36 mg/kg/day for 5 days a week, for 2 weeks and repeated, plus anti-VEGF monoclonal antibody	Only the combination treatment group found free of metastatic disease	68
Subcutaneous human breast cancer xenografts implanted into SCID-mouse–human skin chimaeras	Weekly CTX plus anti-endoglin antibody	Combination treatment was only one effective in suppression of human vessels, and was highly effective in suppressing tumour growth	96
Human soft-tissue sarcoma xenografts in SCID mice	Doxorubicin administered every 3 days and DC101 anti-VEGFR2 antibody twice per week	Most effective and least toxic treatment consisted of low-dose chemotherapy plus DC101 antibody	70
Human testicular germ-cell tumour xenografts	Low-dose cisplatinum on days 14 and 21 plus daily TSP1 or endostatin	Metastatic growth affected only in combination treatment groups	71

Metronomic chemotherapy was used in combination with anti-angiogenic drugs. CTX, cyclophosphamide; i.p., intraperitoneal; MMP, matrix metalloproteinase; P-pg, P-glycoprotein; s.c., subcutaneous; SCID, severe combined immunodeficient; TSP1, thrombospondin 1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

prevent their binding to serum proteins. Clinically relevant concentrations of these vehicles or binding proteins can significantly dampen the anti-angiogenic activity of taxanes, meaning that higher doses of such injectable taxanes would have to be used *in vivo* to induce anti-angiogenic effects[82].

Most of these studies reported no cytotoxic effects, although we have found that if endothelial cells are continuously exposed to a low concentration of drug such as paclitaxel over a 6 day period (replicating metronomic therapy), endothelial cells, but not dermal fibroblasts or tumour cells, undergo apoptosis within about 5 days[78]. This delay in cytotoxicity indicates that the pro-apoptotic effects of low-dose metronomic chemotherapy on endothelial cells might not be direct, but could instead be a secondary result of some other process that is specific to the vascular endothelial cell. This concept is illustrated in FIG. 2.

Two recent studies implicate thrombospondin 1 (TSP1) as a potential mediator of the effects of metronomic cyclophosphamide[33,34]. In one study, 5 days of exposure to low concentrations of various chemotherapeutic drugs caused a marked increase in *TSP1* mRNA and protein levels in vascular endothelial cells *in vitro* (other cells were not tested). TSP1, a component of the extracellular matrix that can also be secreted and found

Table 2 | **Sensitivity of human vascular endothelial cells to metronomic therapy**

Drug(s)	Assay(s)	Results	References
Methotrexate	Inhibition of cell proliferation	Human endothelial cells inhibited by low concentrations of drug (5×10^{-9} M)	47
Paclitaxel	Inhibition of endothelial-cell proliferation, motility and cord/tube formation	Inhibition of endothelial-cell chemotoxins and invasiveness detected after several hours incubation with drug concentrations as low as 10 pM (IC_{50}* = 0.5–4 nM)	77
Vinblastine	Inhibition of proliferation and migration and inhibition of metalloproteinase secretion	Human endothelial cells inhibited by ultra-low concentrations (0.1–1 pM/l); leukocytes, fibroblasts and tumour cells not inhibited	79
Paclitaxel and vinblastine	Inhibition of endothelial- or tumour-cell proliferation in monolayer or tumour spheroid culture (for tumour cells)	Human umbilical endothelial cells inhibited with IC_{50} values in the range of 0.4–0.5 nM; tumour cells inhibited by IC_{50} values in the range of 2–27 nM in monolayer culture, and 3,434–10,084 nM in spheroid culture	65
Paclitaxel, cyclophosphamide and epothilone B	Inhibition of cell proliferation and induction of apoptosis, testing human endothelial cells, fibroblasts and tumour cells	Daily exposure to drugs over 6 days resulted in inhibition of human endothelial-cell proliferation with IC_{50} values in the range of 50–100 pM; IC_{50} values for tumour cells or fibroblasts were generally at least 10 times more than for endothelial cells; induction of apoptosis only detected in endothelial cells	78
Paclitaxel	Inhibition of cell proliferation and endothelial-cell tube formation	Paclitaxel (over three days) selectively inhibits proliferation of human endothelial cells at ultra-low concentrations (0.1–100 pM) with an IC_{50} value of 0.1 pM; six different non-endothelial cell types inhibited at 10^4–10^5-fold higher concentrations (IC_{50} = 1–10 nM); endothelial-cell tube formation also inhibited *in vitro*	80
Paclitaxel and docetaxel	Inhibition of cell proliferation, migration and capillary sprouting	Endothelial cells found to be 10–100 times more sensitive than tumour cells; docetaxel 10 times more effective than paclitaxel	81

In vitro studies were performed with low doses of chemotherapeutic drugs. *The IC_{50} (inhibitory concentration 50%) is the concentration of drug required to inhibit 50% of cell growth.

in the circulation, is a well known endogenous inhibitor of angiogenesis[83,84]. It seems to act primarily by binding to CD36 receptors, which are expressed by endothelial cells[85]. This interaction blocks proliferation and induces apoptosis in endothelial cells[86,87], but would not be expected to occur in CD36-negative cells, such as most bone-marrow-derived haematopoietic stem cells or hair-follicle cells. TSP1 can also bind and sequester VEGF, and therefore block its pro-angiogenic activity[88].

Further evidence to implicate TSP1 as a secondary mediator of the anti-angiogenic properties of metronomic chemotherapy was obtained in experiments that compared the anti-angiogenic and antitumour effects of MTD cyclophosphamide with a continuous daily low-dose regimen of the same drug in wild-type or *Tsp1*-null mice[34]. In these experiments, the drug was administered continuously in the drinking water[19]. The antitumour efficacy (tested on subcutaneously transplanted Lewis lung carcinoma tumours) and anti-angiogenic effects were lost in the *Tsp1*-null mice, but not in the wild-type controls[34,78]. Raghu Kalluri and collaborators have also shown that weekly administration of low doses of cyclophosphamide leads to loss of the antitumour activity of this drug against the B16 mouse melanoma grown in Tsp1-deficient mice[33]. By contrast, the chemotherapy regimen retained its efficacy in mice that were unable to produce either endostatin or tumstatin — two other endogenous inhibitors of angiogenesis[33]. It therefore seems that these molecules are not involved in the anti-angiogenic effects of metronomic treatment with cylophosphamide. TSP1, however, is induced in the melanoma cells and infiltrating host (stromal) cells of the treated tumours[33].

So, metronomic chemotherapy might not necessarily act directly on endothelial cells, but might instead act by inducing endothelial-cell-specific inhibitors, such as TSP1. This could explain why metronomic chemotherapy regimens do not increase the usual harmful side effects of chemotherapy, such as myelosuppression, despite the elimination or shortening of long, drug-free break periods. It is also interesting to note that other anticancer drugs that have anti-angiogenic 'side effects' could also work by a similar mechanism. For example, in 1997 trastazumab, a monoclonal antibody against ERBB2, was implicated to have anti-angiogenic properties[89] , as it inhibits VEGF expression. Furthermore, it can induce TSP1 in tumour cells[90].

As previously discussed, one crucial aspect and advantage of metronomic therapy is that it prevents the repair to the tumour vasculature that occurs between sessions of MTD therapy. What is the basis of this apparently robust and highly specific repair process?

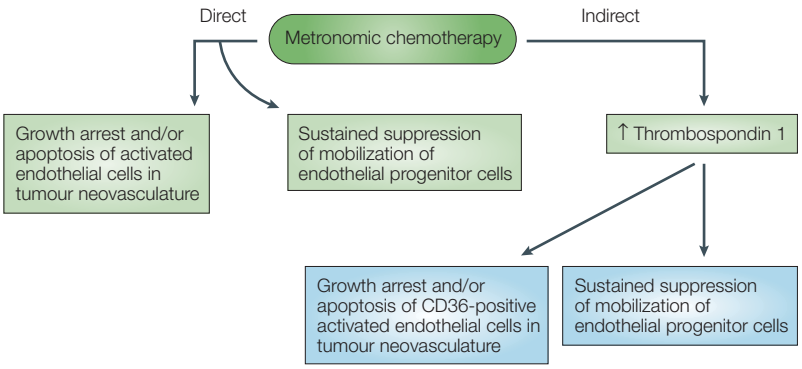

Figure 2 | **Possible mechanisms of the anti-angiogenic basis of metronomic chemotherapy.** There are two routes by which metronomic chemotherapy could lead to growth arrest or apoptosis of endothelial cells in the tumour neovasculature. A 'direct' pathway (left) assumes that activated, differentiated endothelial cells are intrinsically sensitive to low-dose chemotherapy, for which there is some evidence[80–85]; the same might be true for circulating endothelial progenitor cells[17]. The 'indirect' pathway (right) assumes that the levels of metronomically administered drugs are too low to induce growth arrest or apoptosis of endothelial cells. Instead, an endogenous inhibitor of angiogenesis, such as thrombospondin 1, is induced in certain cells by low-dose chemotherapy. This inhibits tumour angiogenesis and vasculogenesis, leading to a reduction in tumour neovascularization in the absence of side effects such as myelosuppression, hair loss, and nausea or vomiting.

Part of the answer might lie in the effects of metronomic chemotherapy regimens on the mobilization, levels and viability of bone-marrow-derived circulating endothelial progenitor cells (CEPs). These cells contribute to some forms of angiogenesis, such as development of the vasculature in early embryonic development, as well as tumour angiogenesis, essentially constituting a form of 'systemic' vasculogenesis and angiogenesis, in contrast to the local division of differentiated endothelial cells in pre-existing vessels. Until 1997, it was thought that all new endothelial cells were derived through the latter process[91], but there are reports that claim that up to 50% of the endothelial cells in newly forming blood vessels come from CEPs[92]. These cells can be mobilized from the bone marrow by growth factors such as VEGF, and then enter the peripheral-blood circulation. There, they can migrate to sites of ongoing angiogenesis and differentiate into mature endothelial cells[20,91,93].

Bertolini *et al.*[17] showed that in immune-deficient mice that were previously injected subcutaneously with human lymphoma cells, the increased levels of CEPs detected in the blood circulation of the mice sharply declined shortly after the mice were treated with a cycle of MTD cyclophosphamide. The number of these cells quickly and sharply rebounded during the drug-free break period, presumably as the result of a compensatory haematopoiesis-like effect[17]. By contrast, when cyclophosphamide was administered at lower doses on a weekly basis or continuously in drinking water, the numbers of CEPs gradually declined, as did their viability, and no compensatory rebound was observed[17]. If CEPs make a significant contribution to tumour angiogenesis — and this remains a point of continuing debate[94,95] — the 'rebound' of these cells during the long break periods after MTD chemotherapy could contribute, at least in part, to the repair process that occurs in the damaged tumour endothelium. This would explain the inability of MTD chemotherapy to inhibit tumour angiogenesis in a sustained, and therefore therapeutically effective, manner.

These studies call into question the use of growth factors as supportive measures to accelerate recovery from the myelosuppression-inducing effects of high-dose, standard MTD or dose-dense chemotherapy regimens. For example, both erythropoietin and granulocyte colony-stimulating factor (G-CSF) are often given to patients to help them recover from anaemia and myelosuppression, respectively, which are induced by MTD chemotherapy regimens. This is because they promote the mobilization of marrow progenitor cells into the peripheral circulation, where they can differentiate into mature white blood cells such as neutrophils or red blood cells, and have been shown to increase the mobilization of CEPs[96,97]. This, in turn, can stimulate vasculogenesis/angiogenesis, leading to tumour growth[98,99], and could provide one explanation for the fact that treatment of patients with recombinant erythropoietin after standard chemotherapy is associated with a worse outcome, in terms of survival, in some clinical trials[100].

Clinical trials of metronomic chemotherapy

Phase II clinical trials have been initiated to test the possible benefits of metronomic chemotherapy regimens — particularly when these are combined with an anti-angiogenic drug. Several of these trials are summarized in TABLE 3. Most of these involve chemotherapy regimens in which cyclophosphamide is administered orally on a daily basis, sometimes for up to 2 years, with no break periods. In some cases, oral low-dose methotrexate is also given on two consecutive days on a weekly basis. The targeted drugs that are used include a cyclooxygenase-2 (COX2)-specific inhibitor such as celecoxib, which is administered on a daily basis, or a humanized anti-VEGF monoclonal antibody (such as bevacizumab), which is administered intravenously every 2 weeks. Celecoxib was selected for inclusion in the trial because of its commercial availability, ease of administration, excellent side-effect profile and putative anti-angiogenic effects[101,102].

The combination of cyclophosphamide and methotrexate has already been tested in a clinical trial in Italy, and has spurred additional trials that are underway[49]. Sixty-four women with progressive, advanced and refractory breast cancer received low doses of oral cyclophosphamide on a daily basis and oral methotrexate was given twice per week. Most of the patients had progressive metastatic disease when the trial began and had also previously received first-, second- or third-line treatments. An overall response rate of 32% was observed, which included two complete responders, 10 partial responders and 12 patients with stable disease lasting 6 months or longer[49]. No high-grade adverse events were reported, despite the fact that many patients had previously been treated with chemotherapy. This compares favorably with the standard third-line chemotherapy regimens used in

Table 3 | **Clinical trials involving metronomic chemotherapy**

Patient population (status of trial)	Drug treatment	References/details
Advanced, refractory melanoma; pilot study of 12 patients (completed)	Daily low-dose (500 mg) oral treosulfan and daily rofecoxib (Vioxx), 25 mg	51
Advanced, refractory prostate cancer; Phase II clinical trial of 32 patients (completed)	Daily low-dose, oral cyclophosphamide (50 mg) and daily low-dose dexamethasone (1 mg)	52
Advanced, refractory breast cancer; Phase II trial of 64 patients (completed)	Daily low-dose, oral cyclophosphamide (50 mg) and oral low-dose methotrexate twice per week	49
Advanced or metastatic ovarian carcinoma (underway)	Daily low-dose cyclophosphamide (50 mg) plus bevacizumab, 10 mg/kg every 2 weeks	A. Garcia, P.I., NCI/CTEP-sponsored multicentre Phase II clinical trial
Advanced, metastatic breast cancer (underway)	Daily low-dose, oral cyclophosphamide (50 mg), oral low-dose methotrexate twice per week (10 mg) and bevacizumab 10 mg/kg every 2 weeks	H. Burstein, P.I., Dana–Farber Cancer Institute
Recurrent and metastatic chemoresistant squamous-cell carcinoma of the head and neck (pilot study; completed)	Daily oral low-dose (2.5 mg) methotrexate and 400 mg celecoxib twice per day	139
Relapsed, refractory non-Hodgkins lymphoma (ongoing)	Oral low-dose (50 mg) cyclophosphamide and 400 mg celecoxib twice per day	140
Metastatic renal cell carcinoma (completed) celecoxib twice/day	Daily oral low-dose (50 mg) cyclophosphamide and 400 mg	141
Hepatocellular carcinoma	Oral low-dose (50 mg) cyclophosphamide and 400 mg celecoxib twice per day	E. Bergsland, P.I., University of California at San Francisco
Refractory solid tumours in children and adults (completed)	Daily low-dose oral cyclophosphamide for 3 weeks followed by daily low-dose oral etoposide for 3 weeks, which is repeated chronically, combined with daily oral low-dose thalidomide and daily oral low-dose celecoxib	138
Pancreatic cacrinoma	Oral low-dose (50 mg) cyclophosphamide and 400 mg celecoxib twice per day	E. Bergsland, P.I., University of California at San Francisco
Advanced metastatic breast cancer	Daily low-dose (50 mg) oral cyclophosphamide and oral low-dose methotrexate (2.5 mg) twice weekly, dalteparin (Fragmin) 5,000 IU s.c. daily and oral preduisone (5mg daily)	K. Pritchard (P.I.) *et al.* Toronto Sunnybrook Regional Cancer Centre

IU, international units; NCI/CTEP, National Cancer Institute Cancer Therapy Evaluation Program; P.I., Principal Investigator; s.c., subcutaneous.

this treatment setting, at least in terms of toxicity. The estimated cost of this outpatient therapy was about US$10 per month[49].

In another recently reported trial, Glode *et al.* treated 32 patients with advanced androgen-independent metastatic prostate cancer. These patients received daily oral doses of cyclophosphamide and dexamethasone[52]. Dexamethasone, in addition to other properties, has been reported to have some anti-angiogenic effects[52]. In this small study, almost 70% of the patients showed a decrease in serum prostate-specific antigen levels of 50% or more. Although such preliminary results are encouraging, they need to be confirmed in much larger, controlled and prospective clinical trials[103].

It is also very difficult to determine conclusively whether these therapies have anti-angiogenic effects that contribute to their putative antitumour efficacy. In this regard, Colleoni *et al.* reported that serum VEGF levels declined in patients who responded to therapy[49]. Bertolini *et al.* reported a reduced number of bone-marrow-derived CEPs in the blood of patients with lymphoma and breast cancer who received daily low-dose cyclophosphamide therapy[104], similar to that observed in patients with rectal carcinoma who are treated with the anti-VEGF monoclonal antibody bevacizumab[105]. Nevertheless, the therapies obviously have other effects that contribute to their efficacy — this is especially true for drugs such as celecoxib and dexamethasone. For example, COX2, which is inhibited by celecoxib, can be expressed by tumour cells as well as by activated endothelial cells[101]. It is also known that low-dose chemotherapy, can stimulate the immune system in some cases,[106,107], making it a potentially useful addition in combination with tumour vaccines or other types of immune-therapy approaches. Indeed, some studies indicate that metronomic chemotherapy using cyclophosphamide can increase the efficacy of immunotherapeutic vaccines in preclinical models[108,109].

Metronomic therapy might also have some direct effects on tumour cells, such as induction of cell differentiation, although there is not yet any evidence for this. Continuous-chemotherapy regimens could also

Table 4 | **Metronomic chemotherapy in paediatric oncology**

Disease	Drug(s)	References
Lymphoma	'CHOP' (cyclophosphamide, doxorubicin, vincristine and prednisone)*	41
Acute lymphatic leukeamia	Methotrexate and 6-mercaptopurine‡	42,45
Wilms' tumour	Vincristine§	44
Rhabdomyosarcoma	Vinblastine and actinomycin, or vincristine, actinomycin and cyclophosphamide‖	43

*Induction remission: (days 1–47), vincristine, 1.5 mg/m^2, weekly for 7 weeks (maximum dose, 2.0 mg), cyclophosphamide, 750 mg/m^2 intravenously on days 1, 22 and 43; doxorubicin (adriamycin) 40 mg/m^2 intravenously on days 1, 22, and 43; prednisone 40 mg/m^2, oral, days 1–28, and then days 43–47. ‡Methotrexate 20–50 mg/m^2/week for up to 2 years and 6-mercaptopurine, 50–75 mg/m^2 daily for up to 2 years; all protocols, but this is the range. §Vincristine: same dose for treatment of patients with lymphoma (1.5 mg/m^2) weekly for 10 weeks and then on weeks 12, 15 and 18. ‖Vincristine 1.5 mg/m^2, weekly for 7 weeks as part of a combination chemotherapy regimen; for example, vincristine, actinomycin and cyclophosphamide, which may be given weekly for 13 weeks, with a 4 week break, and weekly for 8 weeks, followed by a 3 week break and then 12 of the next 17 weeks.

prevent a rebound in tumour-cell division known as 'repopulation', which can take place during the rest periods between cycles of MTD chemotherapy. Repopulation kinetics can become increasingly more aggressive with successive cycles of MTD pulsatile chemotherapy[110], so shortening or eliminating the drug-free break periods might prevent this 'kinetic drug resistance'.

Several successful paediatric chemotherapy approaches resemble metronomic therapy, as discussed above, in that they involve daily administration of low doses of cytotoxic drugs over prolonged periods of time, as so-called 'maintenance' therapies, and have minimal toxicity (TABLE 4). Paediatric oncologists have shown that daily adminstration of cyclophosphamide along with weekly administration of *Vinca* alkaloid drugs, such as vincristine or vinblastine, is effective in treating patients with diseases such as neuroblastoma[111].Weekly administration of *Vinca* alkaloids is also a key component of therapy for patients with Wilms' tumour and rhabdomyosarcoma. Paediatric 'CHOP' (cyclophosphamide, adriamycicin, vincristine and prednisone) therapy, which is used to treat patients with non-Hodgkin's lymphoma, is also conceptually similar to metronomic therapy. Aggressive fibromatosis can also be controlled with low-dose vinblastine and methotrexate treatment[112]. Other low-dose chemotherapy regimens that are being tested in children, with some hints of success, include low-dose cyclophosphamide treatment and low-dose vincristine therapy to treat infants with localized, unresectable neuroblastoma[111].

The oral fluoropyrimidine[113] agent, UFT (uracil plus tegafur) — a prodrug mixture that is metabolized to 5-FU and two other metabolites, γ-butyrolactone (GBL) and γ-hydroxybutyric acid (GHB) — represents another chemotherapeutic that might be applied metronomically[74,111,114]. This drug is approved for treatment of certain cancers in Japan and throughout Europe, but not in the United States[115]. Recent randomized Phase III ADJUVANT clinical trials have been undertaken in patients with resected early-stage NSCLC of the adenocarcinoma variety. These patients were given daily low doses of oral UFT for 2 years, resulting in both a survival benefit and very little toxicity, despite the chronic nature of the treatment[116]. 5-FU, GBL and GHB have all been shown to have anti-angiogenic activity in an *in vivo* assay[74,117] , as well as to inhibit growth and migration of endothelial cells in culture[74,117] — especially when they are administered continuously at low doses[74]. Could the anti-angiogenic effects of this drug contribute to its clinical efficacy? Could this efficacy be increased by combination therapy with a targeted anti-angiogenic drug? Further studies are necessary to answer these questions. The trial of UFT in patients with lung cancer, however, shows the potential of oral long-term metronomic chemotherapy as an adjuvant therapy to treat patients who could have microscopic, early-stage recurring tumours.

Further experiments are required to determine whether other chemotherapeutics that can be orally administered on daily schedules for extended periods of time, such as the topoisomerase enzyme inhibitor etoposide (VP-16) or the alkylating agent temozolamide[118], also have anti-angiogenic effects[119] that contribute to their antitumour efficacy. There are several other situations in which prolonged oral administration (4–5 weeks) of relatively low doses of chemotherapeutics is already in use, such as administration of etoposide[37,120], razoxane[121] or temozolamide[122,123] to treat malignant melanoma and non-Hodgkin's lymphoma. These are palliative-like regimens that are less toxic and are sometimes used to treat elderly patients, who are less able to cope with toxic MTD chemotherapy. It will be important to determine if anti-angiogenic effects contribute to the efficacy of such protocols — there is already some preliminary evidence that this is the case[119,121]. These studies could also accelerate the development of oral taxanes and other microtubule inhibitors for metronomic therapy[124]. Another approach involves the use of injectable chemotherapeutics that are incorporated into endothelial-targeted liposomes to increase the half-life of the drug in the circulation[125].

Other types of metronomic therapy

The metronomic approach is not only used with chemotherapy, but also with radiation therapy. Sometimes, radiotherapy is administered at lower than normal doses, known as 'hyperfractionated radiation'[92]. In this regard, Garcia-Barros *et al.* have shown that the antitumour effects of irradiation can be mediated through a primary event that involves damage or destruction of the neovasculature of a tumour, followed by the death of tumour cells that surround the affected vessels[92]. This could help to explain the increased therapeutic efficacy of combining radiation therapy with endothelial-cell-specific anti-angiogenic drugs such as angiostatin or anti-VEGF antibodies[126,127].

The cytokine intereferon-α (IFN-α) also has anti-angiogenic effects, and is used effectively to treat paediatric patients with haemangiomas or giant-cell tumours when administered in small daily doses over prolonged periods of time[128,129]. This might be considered to be another example of the efficacy of anti-angiogenic

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Administration of certain anticancer drugs, such as tamoxifen, for prolonged periods — even as long as 3–5 years. This form of treatment is usually used to treat microscopic metastatic disease, after surgical removal of the primary tumour, or sometimes for treatments of a primary tumour, in which case it is called neoadjuvant therapy.

metronomic therapy. Preclinical studies from Isiah Fidler's group have also shown that metronomic administration of IFN-α therapy is significantly more effective as an anti-angiogenic and antitumour treatment strategy in mouse xenograft models of cancer[130]. They have shown that daily administration of 10,000 units of IFN-α was more effective than 70,000 units administered once a week as an anticancer therapy[130].

Future directions

Although we have explained the potential benefits of metronomic chemotherapy regimens — especially when used in a combination-therapy context with targeted anti-angiogenic agents — there are several significant challenges that must be overcome to increase the chances of success in the clinic. Foremost among these is the current empiricism associated with trying to determine the optimal dose and schedule for administration of chemotherapeutics. Other medical subspecialties have defined minimally inhibitory concentrations, such as those used to treat patients with infectious diseases or epilepsy.

It seems that there are two main approaches to administering chemotherapy. Perhaps a useful analogy is to compare these approaches to radio airwaves. 'Amplitude modulation' (AM) involves increasing the dose, but this requires increasing the time between the doses, whereas 'frequency modulation' (FM), involves decreasing the amplitude (the unit dose/time). There are some obvious advantages to FM, such as reduced acute toxicity and the ability to combine the drug with targeted therapeutics for prolonged periods. The challenge therefore is to find the smallest dose that will control the target cells and then the most frequent dosing that will maximize this control. This type of problem is obviously not unique, as many molecularly targeted drugs do not produce their maximum therapeutic effects at the MTD, and some do not even have dose-limiting toxicities[131,132]. Similarly, determining the biological activity of such agents in the absence of acute tumour regression can be difficult.

These problems might eventually be overcome through the discovery and application of novel molecular or functional surrogate markers, to guide dose selection and to monitor antitumour activity. The same could be the case for metronomic chemotherapy. So, detecting changes in levels of circulating TSP1 levels in serum or plasma after administration of various low doses of chemotherapeutics might be useful in determining the optimal low dose for a drug such as cyclophosphamide[34]. Another possibility is the application of functional imaging approaches, such as those used to detect changes in tumour blood flow. For example, Walter Wolf and colleagues recently reported that administration of docetaxel on either a once-every-week schedule, or a once-every-3-weeks schedule, resulted in reductions in tumour blood flow in several patients with breast cancer, as determined by dynamic-contrast magnetic-resonance imaging. Moreover, there seemed to be a strong correlation between this change and tumour response[133]. This is strikingly similar to the results of a clinical study involving administration of an anti-angiogenic drug called PTK787 — a small-molecule antagonist that blocks several receptor tyrosine kinases, including VEGFR2 in patients with colorectal carcinoma[134]. So, chemotherapeutics could have significant antivascular properties, which in some cases might be their primary effector function in terms of tumour destruction.

Another promising approach to determining the optimal low dose for a given metronomic chemotherapy regimen is evaluation of the activities of CEPs or circulating endothelial cells (CECs). As previously discussed, CEPs mobilization from the bone marrow into the peripheral circulation is strongly inhibited by low-dose cyclophosphamide, as is CEP viability[17]. So, there could be a direct relationship between the relative efficacy of different (low) doses of metronomic chemotherapy and the ability of these doses to reduce levels of CEPs in the peripheral circulation (Y. Shaked & R.S.K., unpublished observations). Furthermore, assays have been developed to detect the presence of CECs and CEPs in blood samples, such as by performing RT-PCR to detect the mRNA for the vascular endothelial cell-adhesion molecule VE-cadherin[135]. It is therefore encouraging that infusion of bevacizumab in patients with rectal carcinoma was recently found to lead to a rapid decline of both CECs and CEPs in the peripheral blood, as these assays might be exploited not only to monitor anti-angiogenic drug or treatment activity, but to help determine the optimal dose.

A second challenge is the prospect of delayed side effects, including secondary neoplasms, when administering protracted regimens of DNA-damaging agents or other types of genotoxic agents, although it might be argued that these are not a serious concern when treating patients with advanced-stage cancers. But it is certainly a concern in adjuvant-therapy settings for patients with early-stage disease, many of whom are already cured.

As for toxic side effects, although these might be delayed, they might nevertheless be significant. We have found that mice treated with protracted daily oral low-dose cyclophosphamide can eventually develop lymphopaenia[35]. Similarly, patients treated with extended low-dose temozolamide for successive cycles of 6 weeks with 2-week breaks can develop immunosuppressive T-cell lymphopaenia[136]. In this regard, the minimal-toxicity profile observed in patients with early-stage resected NSCLC who were given low doses of UFT every day for 2 years is encouraging — grade 3 toxic effects occurred in only 10/482 (2%) of patients[116]. This is particularly important because anti-angiogenic drugs and metronomic chemotherapy regimens might work best, and sometimes only, in patients with low-volume disease burden. This is true for almost all anticancer drugs and treatment strategies, for various reasons. These include reduced drug access to larger tumours, as well as reduced oxygen levels (hypoxia), which can attenuate the toxic effects of radiation or chemotherapy. It might be possible to treat early-stage disease with long-term combination therapies, but this clearly necessitates the use of non-toxic and less expensive drugs.

Hopefully, some of the clinical trials that are underway, especially those that are prospective and randomized[103], will better indicate the potential promise of this therapeutic strategy — particularly when such metronomic chemotherapy regimens are integrated with new molecularly targeted drugs. In particular, there is a need to learn more about which chemotherapeutics are the most effective for metronomic dosing regimens, what combinations and sequences might be best to use, and what mechanisms of resistance might develop over time[75,76]. Furthermore, it will be important to determine the types of cancer that might be the most responsive to these therapeutic approaches. There is already some data available for the treatment of breast cancer[137] and tumours of the central nervous system[138] using this approach. As scientists carrying out basic research perform more preclinical studies this approach and begin to work more closely with clinical investigators that are leading metronomic chemotherapy-based clinical trials, there should be significant progress towards answering these questions over the next few years.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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