From total empiricism to a rational design of metronomic chemotherapy phase I dosing trials

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'Metronomic chemotherapy' represents a novel anti-angiogenic strategy whereby low-dose chemotherapy is utilized in a continuous fashion in order to target tumor endothelium. There are many potential advantages of this strategy and clinical trials are already underway. However, although the scheduling of metronomic chemotherapy is relatively unequivocal, metronomic dosing principles are at present poorly defined. Arbitrarily, 10–33% of the maximum tolerated dose comprises 'the dose range'. We argue that this is too empirical and propose a set of phase I metronomic chemotherapy dosing strategies based on a principled approach which may help to reduce the problem of empiricism in dosing for metronomic chemotherapy trials. ***Anti-Cancer Drugs*** **17:113–121 © 2006 Lippincott Williams & Wilkins.**

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Introduction

The objective of traditional chemotherapy is complete tumor eradication. Whether that be of micrometastases, i.e. adjuvant treatments, or voluminous disease (e.g. testicular cancer), it is based on the precept that tumor cells represent the prime target of chemotherapy and eradication can only be achieved through the repetitive administration of a conventionally derived maximum tolerated dose (MTD). The influential work of Skipper *et al.* [1] four decades ago established the MTD paradigm for cancer therapy, using the log-dose survival curve as a model of tumor response towards escalating doses of drugs. Since high doses of cytotoxic agents were employed, adverse effects were inevitable and to ameliorate such effects chemotherapy protocols usually consisted of several cycles of intense drug exposure punctuated by long rest periods to allow susceptible non-neoplastic organs such as the bone marrow or gut to recover. While this paradigm has worked well for some hematological malignancies and germ cell cancers, for the majority of solid adult malignancies, neither complete tumor eradication nor sustained regressions are often achieved. There are several reasons which may account for this phenomenon. The MTD paradigm was developed using a model which was based on synchronized cell culture systems in which chemotherapy was optimized to affect all cells growing in culture. Such a model is unlikely to reflect the nature of most adult cancers, which consist of heterogeneous cell populations, where only cells in cycle are likely to be affected by chemotherapy. Furthermore, genetic and epigenetic variations within a heterogeneous population of tumor cells result in diversity in drug sensitivity. These properties eventually confer refractoriness towards conventional chemotherapy [2]. In addition, the MTD model does not take into account the importance of tumor organization, which has been shown to contribute towards the development of multicellular drug resistance [3].

An alternative strategy for the management of adult solid malignancies refractory to chemotherapy is to manage cancer as a chronic, but stable disease state, where the total tumor burden is kept at the lowest possible level [2]. Indeed, it has long been known that survival need not necessarily be incompatible with the presence of tumor, as long as its growth is controlled. Crucially, some studies have shown that patient survival depends more on a cytostatic effect of chemotherapy rather than on the ability to induce tumor remission [4,5]. Secondly, a new paradigm for chemotherapeutic dosing is based on the targeting of tumor vasculature rather than tumor parenchyma using low-dose, long-term continuous chemotherapy [6–8]. The term 'metronomic dosing' was first used by Hanahan *et al.* [8] in describing such a protocol, based on low doses and regularly spaced dosing schedules of cytotoxic agents without any extended rest periods. As such, metronomic dosing of chemotherapy is a novel therapeutic strategy which embraces the new chemotherapeutic paradigms described above and hence represents a radical departure from traditional notions of chemotherapy.

In considering metronomic chemotherapy as a strategy for treating cancer, several fundamental points need to be highlighted. First, in targeting tumor endothelium

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instead of tumor cells *per se*, such a strategy utilizes cytotoxic agents in a predominantly anti-angiogenic fashion. This apparent shift in concept was largely prompted by experimental studies which surprisingly showed that tumor angiogenesis can be inhibited by cytotoxic agents, particularly when administered at low and frequent doses. This type of dosing seems to be more effective than high doses followed by extended rest periods [6]. The rationale for this approach lies in the characteristics of tumor endothelium which, in contrast to the endothelium of quiescent and fully differentiated blood vessels in adults, consists of activated cells that proliferate rapidly and hence are relatively susceptible to the pro-apoptotic effects of cytotoxic agents [9]. In retrospect, the inhibitory effects of chemotherapy on tumor vascularity had long been known by oncologists, but notably this was regarded as an unwanted side-effect rather than a property of chemotherapy which could be exploited, based on the erroneous notion that such anti-vascular effects might compromise delivery of chemotherapy to tumor parenchyma [10]. Studies on combinations of anti-angiogenic agents with chemotherapy or ionizing radiation have shown that inhibition of tumor angiogenesis can enhance the effects of chemotherapy and ionizing radiation through various mechanisms. These include decreased leakage of plasma proteins from tumor vessels and reduced intra-tumoral pressure, resulting in increased delivery of oxygen and therapeutic agents to tumors [11,12], and prevention of repair of radiation or cytotoxic-induced damage to endothelial cells [13]. In spite of the fact that the beneficial anti-angiogenic property of cytotoxic agents was first reported as far back as the early 1980s [14], it is only recently that such a property has been acknowledged and regarded with the degree of seriousness that it warranted. Presently, most common anti-cancer chemotherapeutic agents, belonging to all major classes, have been shown to be capable of inhibiting angiogenesis (reviewed in [15]). Miller *et al.* [15] also suggested some criteria by which a cytotoxic agent might be considered to possess true anti-angiogenic activity.

Secondly, since tumor vasculature is in direct contact with the systemic circulation, therapeutic agents have efficient and direct access to their targets. This is in contrast to conventional chemotherapy that targets tumor parenchyma, which is located within the interstitial compartment of the body and is poorly perfused. Moreover, since tumor blood vessels are chaotic and irregular, and contain transport channels which operate in a dysregulated and haphazard manner, drug delivery to neoplastic cells within a tumor is unpredictable and uneven [12]. The efficient access afforded by metronomic chemotherapy to the cellular target (i.e. activated endothelial cells) however, allows the concentration of drugs needed for optimal efficacy to be reached relatively easily and, perhaps more importantly, it also improves the ability to achieve sustained drug concentrations required for prolonged inhibition of angiogenesis.

Furthermore, the risk of acquiring drug resistance associated with the long-term use of metronomic chemotherapy is likely to be significantly lower than for conventional chemotherapy, since the target of therapy is tumor endothelium, which is host-derived and genetically stable, unlike tumor tissue [16]. It needs to be acknowledged however, that no drug is completely free from the risk of drug resistance. Indeed, different classes of selective angiogenic inhibitors have different levels of susceptibility towards acquiring drug resistance. For instance, factors such as endostatin and angiostatin, which inhibit the growth and functions of activated endothelial cells directly and independently of the tumor microenvironment, are less likely to cause resistance compared with indirect inhibitors such as drugs or antibodies which inhibit the actions of tumor-derived pro-angiogenic growth factors [e.g. vascular endothelial growth factor (VEGF) or basic fibroblast growth factor] or their receptors [15,17]. The mechanism of action of indirect angiogenic inhibitors is fundamentally influenced by the tumor and its microenvironment – a property which makes it more susceptible to the acquisition of chemoresistance. A further important consideration relating to the tumor microenvironment is that there is some recently emerging evidence pointing to the ability of some anti-angiogenic treatments to cause cytogenetic abnormalities in stable endothelial cells. In an elegant study in tumor xenografts, the induction of aneuploidy was demonstrated in tumor endothelial cells compared with endothelial cells from other tissues of the same animal [18]. How relevant this artificial system is (human cancer cells and their effect on murine endothelial cells) to the clinic is hard to gauge, but it may offer an explanation for the observed acquired resistance and progression of cancers that initially respond to anti-angiogenic treatment in the clinical setting. Nevertheless, it is still assumed that the overall risk of acquiring drug resistance during anti-angiogenic treatment is likely to be significantly lower and its onset delayed compared with traditional cytotoxic therapy [16].

The final notable point with regard to metronomic chemotherapy is that the latest clinical development in biological anti-angiogenic agents has shown that the greatest efficacy in survival and response (clinical endpoints) is achieved in combination with chemotherapy, especially those which are based on microtubule inhibitors (e.g. paclitaxel and vinblastine). What is not clear is whether the combination needs chemotherapy dosed conventionally, i.e. MTD, or metronomically. Data from recent clinical trials may help to resolve this question. Irinotecan, fluorouracil and leucovorin (IFL) in a high-toxicity bolus regimen in metastatic colorectal cancer was inferior to a lower-toxicity infusional chemotherapy

schedule [19]. This same high-dose regimen failed to show improved survival in the adjuvant setting compared with low-toxicity infusional chemotherapy [20,21]. Nevertheless, the efficacy of the high-toxicity IFL regime was significantly enhanced by the addition of an anti-angiogenic agent [22]. The likelihood is that lower toxicity chemotherapy in combination with the same anti-angiogenic agent may confer an even greater advantage with less toxicity. Studies are ongoing in this area. The question we ask, however, is this: Why should the chemotherapy be dosed conventionally at all? Perhaps all that is needed for the endothelial cell to exhibit greater susceptibility to the biological anti-angiogenic agent is the maintenance of a continuous low-level stress induced by a cytotoxic agent. *In vitro* and preclinical animal data certainly seem to lend some credence to this notion [23,24].

For further in-depth details of the history, development and latest concepts on metronomic dosing of chemotherapy, we refer the reader to a recent extensive review [25]. In this short review we try to come to grips with the inherent weakness of metronomic dosing in the practical sense, and we propose a solution based on our understanding of the preclinical work developed by us and others.

The empiricism of metronomic dosing

Phase I cancer dosing may be an arcane and hopefully one day obsolete practice; however, as the difficulties being experienced by proponents of biological treatments in defining dose for further trials have shown, one is more likely to achieve widespread acceptance of dose and schedule if these can be based on reproducible standards. The dose-finding element of a conventional phase I study in cancer chemotherapy, although fundamentally empirical, is now underpinned by a number of principles that are, more or less, widely used. The design issues involve selecting a starting dose, defining rules for dose escalation or de-escalation between patient cohorts, determining the number of patients to be treated in each cohort and defining an endpoint [26]. The classical endpoint in chemotherapy drug dosing is the dose-limiting toxicity (DLT). The definition of what DLT is and how MTD is derived also follows a set of principles. (A review of this field is beyond our scope; the interested reader should see [26] for a detailed discussion.) This methodology has been so inculcated in the oncologist's training that the development of a number of pleiotropic biological agents which probably work through different principles than those of chemotherapy were still and sometimes are still developed with the MTD mentality (*cf.* interferon/interleukin). Further skeptics may point out that extreme empiricism is already leading to clinical results [27–29]. Why should we try to understand how a flat dose of 50 mg/day of cyclophosphamide works? Furthermore, how does this dose even relate to the preclinical metronomic schedules of Bocci *et al.* [30] of about 25 mg/kg/day, Shaked *et al.* [31] of about 20 mg/kg/day and Pietras and Hannahan [32] of about 10 mg/kg/day, all given per-os in drinking water, and Hamano *et al.* [33], 170 mg/kg given as an every-6-day i.p. injection, and Browder *et al.* [6], 170 mg/kg s.c. every 6 days.

The studies listed above prompt a number of questions. Which dose/schedule is preferable? Which one is more effective? Does a flat dose of cyclophosphamide really work in the clinic? The simple reduction of VEGF in patients with breast cancer [27] or prostate-specific antigen in patients with prostate cancer [28,29] in phase II trials is encouraging, but not convincing. We are still far from having definitive data for these treatments from correctly powered phase III clinical trials. In fact, our argument, that there is a need to try and standardize dosing and establish that a metronomic dose range has anti-angiogenic properties, is further strengthened by the very recent contradictory findings of the usefulness of bevacizumab in breast cancer treatment when combined with either of two of the most effective chemotherapy agents for this condition. Combination with capecitabine has failed to demonstrate an expected clinical endpoint for an anti-angiogenic treatment [increase in time to progression (TTP)] [34] compared to the chemotherapy agent alone. The latest data from the E2100 trial (abstracted in [35]) however, seem to indicate that this endpoint (i.e. increase in TTP) has been achieved when bevacizumab has been combined with weekly paclitaxel. These data, albeit in conventional dosing schedules, should cause pause for thought. They raise the possibility that unless we do try to understand these mechanisms, empirically combining agents and strategies may inhibit beneficial effects rather than enhance them.

The issues we raise about metronomic chemotherapy are just an extension of the debate that already has now been joined about the appropriate dose ranging-finding studies for the newer biologicals (e.g. vaccines, antibodies, anti-angiogenic agents, etc.). Many investigators of immunological approaches to cancer find that the classical phase I endpoints are unlikely to meet the development needs of these agents. There is therefore a persistent call for adequate and reproducible surrogate biological endpoints to be introduced. There is very little consensus however, at present on how to go about things. If the development of monotherapy is therefore so difficult, combination treatments will be doubly so! Metronomic chemotherapy will not be immune to this debate. We would argue that there are already enough data to allow for a principled approach to metronomic dosing.

The starting point for conventional dosing is empirical (e.g. 1/10 of the LD_{50} of a preclinical animal-based toxicity study). From there on, a dose for human use is defined based on phase I study principles. The starting

dose for metronomic chemotherapy is equally empirical and no further effort to define this dose is usually made. The variability of clinical response and the concern about reproducibility will induce a measure of uncertainty. The issue of correlating responses amongst different conventional dosing schemes (e.g. taxanes in ovarian cancer) is complicated as it is and it will be even more difficult if the metronomic dosing scheme has little or no rational basis to justify its selection. Given the bewildering number of potential anti-angiogenic agents, the further development of combination treatments will become even more controversial.

Standards for phase I metronomic dosing studies

Reflecting conventional principles, the metronomic phase I trial methodology that we propose is based on the following standards: (i) choice of initial dose and dosing schedule, (ii) dose ranging, and (iii) endpoint.

Choice of initial dose and metronomic dosing schedule (MetS)

There is no accepted definition of what constitutes a metronomic dose. However, the majority of clinical trials have attempted to arbitrarily define a dose range of 10–33% of the MTD as being representative of the metronomic dose (reviewed in [36]). This approach may be a useful starting point for chemotherapy agents in current clinical use, but misses the point of scheduling, given that most agents are scheduled in conventional 'high-dose packages'. For example, 10% of a 3-weekly conventional dose given daily could lead to a 2-fold increase in total dose which may or may not produce toxicity. Accumulation of metabolites in these schedules would also have to be studied. For novel agents that may be even more useful given the drive towards oral bioavailability, we suggest that MetS should be developed in parallel with conventional dosing schedules. Therefore, the starting point can be the same starting point for conventional studies such as the 1/10 LD_{50}, but preclinical studies should include continuous dosing as one of the schedules. While conventional dosing schedules can have bewildering complexity, we would argue that only two schedules would be relevant for metronomic dosing, and these are daily oral schedules and continuous infusion schedules administered either i.v. or s.c. Other common routes of delivering drug therapy such as transdermal, transmucosal, transrectal or inhalational administration are not appropriate for systemic chemotherapy or for protracted treatment schedules. Pharmacokinetic studies of drug and metabolites given in these continuous schedules should be undertaken as per the conventional approach.

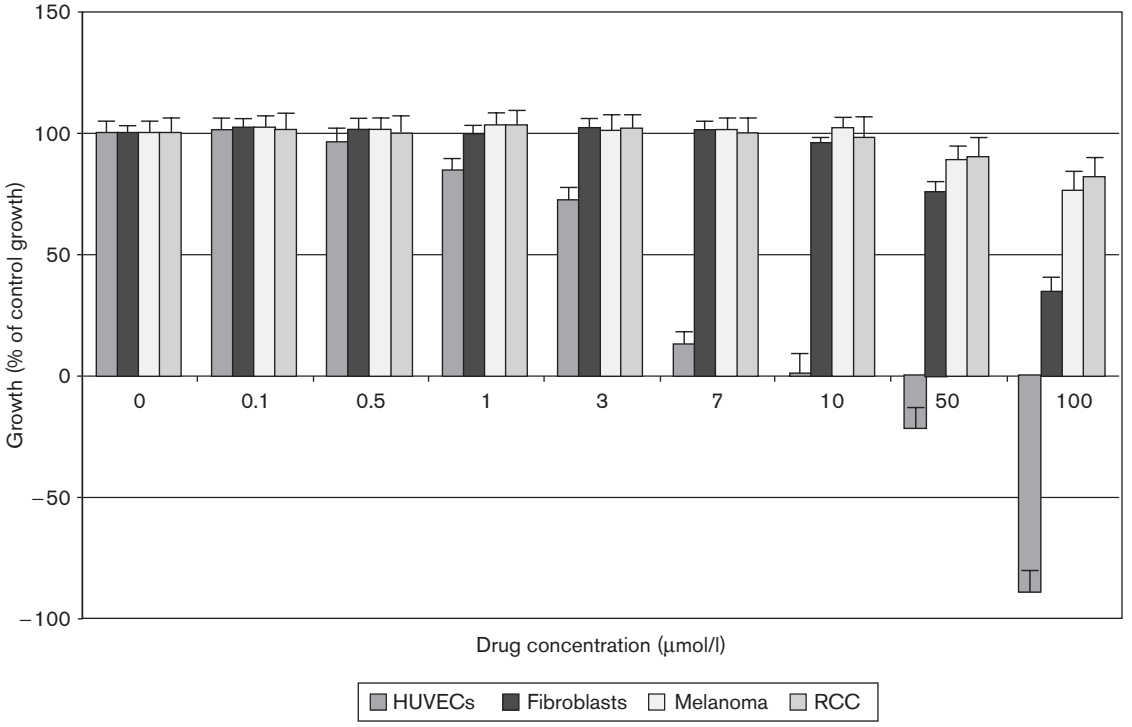
Given the current emphasis on metronomic chemotherapy in its application as part of a combined treatment regimen with other molecularly targeted treatments [e.g. VEGF receptor (VEGFR)-2 inhibitors, endothelial growth factor receptor inhibitors and tyrosine kinase inhibitors] [25,36], we acknowledge the need to incorporate selective inhibitors of angiogenesis into the metronomic protocol in subsequent phases of the study. Such phases would be based on the relevant metronomic doses as determined by the initial MetS study, and should also allow an assessment of synergy of anti-angiogenic activity between the cytotoxic drug and anti-angiogenic agent.

Dose ranging to derive the metronomic dose (MetD)

We would argue that dose ranging is essential given that there is very good evidence *in vitro* that different agents have different capacities to inhibit the growth of endothelial cells at different doses in relation to their MTD. Work from our laboratory (Figs 1 and 2) clearly shows that for similar exposure time and conditions the effects of an alkylating agent (temozolomide) are different to those of an anti-mitotic agent (estramustine) on human umbilical vein endothelial cell (HUVEC) growth. Although both drugs appear to have significant anti-endothelial effects at similar doses (i.e. 3–7 μmol/l), correlation with their MTD using known data from previous pharmacokinetic studies based on conventionally dosed schedules suggests that each drug is active at different proportions of its MTD. For instance, pharmacokinetic data from phase I clinical trials of oral and i.v. dosing schedules of estramustine suggest that peak plasma levels of 3–7 μmol/l are of the order of 10–33% of the MTD [37,38]. In contrast, similar pharmacokinetic studies on temozolomide suggest that levels of 3–7 μmol/l are of the order of 5–10% of the MTD [39]. Using a similar approach, corresponding figures for other cytotoxic agents can be derived. For instance, paclitaxel was shown to be capable of inhibiting the growth of endothelial cells at below 100 pmol/l [40]. Data from pharmacokinetic studies involving different conventional schedules of paclitaxel suggest that such peak plasma levels are well below 0.1% of its MTD [41].

The apparent anti-proliferative effects of such drugs given on a purely metronomic-like schedule on HUVECs appear to be specific as the growth of other relevant cell types, including fibroblasts, melanoma and renal carcinoma cells (RCCs), are not affected. The effects on HUVECs as determined by two independent cytotoxicity assays appear to be non-apoptotic (Fig. 3), suggesting an underlying anti-proliferative mechanism. Nevertheless, even at these low drug doses we have found a clear dose–response curve as can be seen in the presented data. We would therefore propose that clinically relevant doses that can be given in a metronomic fashion should correlate with doses with the greatest effects on the endothelium as determined through *in vitro* cell culture experiments. Whether this is a pure anti-angiogenic effect or a pleiotropic affect is probably irrelevant as long

Fig. 1



Endothelial cell-specific growth inhibition at low-doses following continuous exposure to estramustine. Experiments were conducted in quadruplicate and repeated at least twice. All cells, i.e. HUVECs, human fibroblasts, human melanoma and human RCC cells, were under passage 6 and grown as monolayers in 96-well plates and treated with the agent for 4 days, with daily replacement and replenishment of media. Cell proliferation assay performed via MTS assay with background-subtracted absorbance at 490 nm determined hourly for up to 4 h. Baseline cell growth = [(cell no. of test at 4 days − cell no. of test prior to treatment)/cell no. of test prior to treatment] × 100%. The baseline growth of control, untreated cells (i.e. control) is taken as 100% and growth of treated populations of cells is expressed as percentage of this control growth (mean ± SE). Reproduced with permission from Maraveyas A, Lam T, Hetherington JW, Greenman J. Can a rational design for metronomic chemotherapy dosing be devised? *Br J Cancer* 2005 **92**:1588–1590. Published by Nature Publishing Group

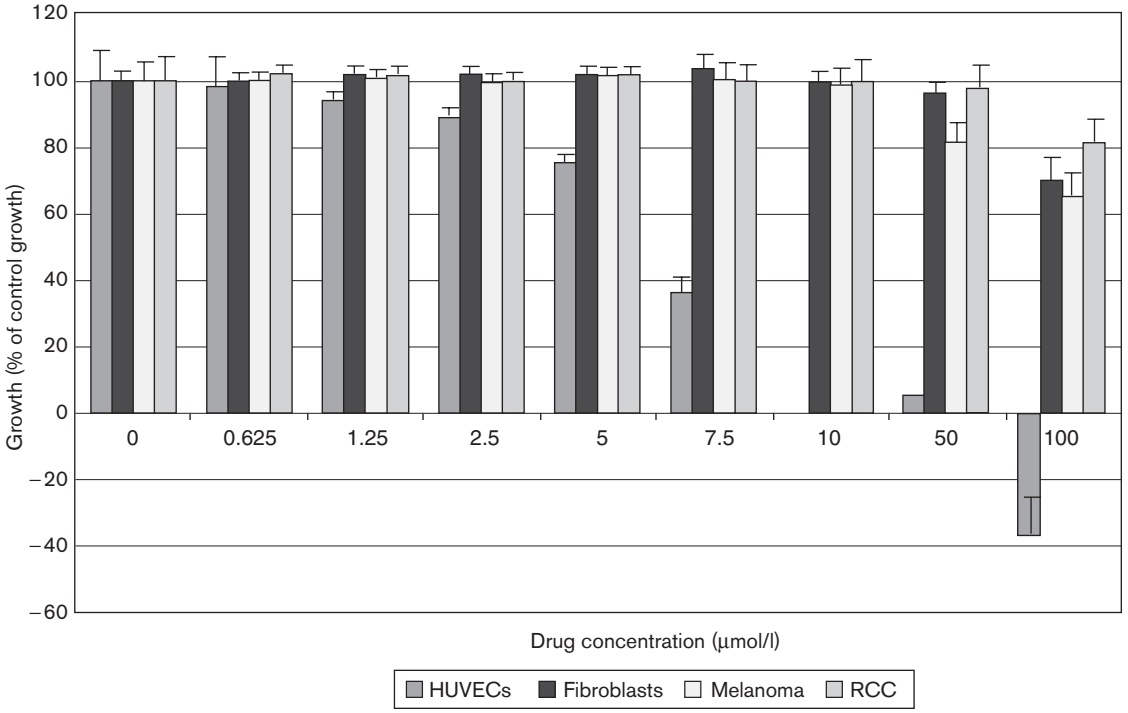
as the dose with the highest potential to deliver this result can be given. We have noted that these observations hold true for all agents we have studied (unpublished data); we therefore argue that efficacy may be compromised by not trying to optimize dose and the approach we have presented above may provide the basis of a rational approach towards defining the MetD.

It has to be noted that the use of in-vitro angiogenesis models based on HUVECs or any other endothelial cell type to represent tumor endothelium is not without its problems. There is a valid argument in using models based on cells of microvascular origin (e.g. human microvascular endothelial cells) to more accurately reflect the microvascular nature of angiogenesis in-vivo. No in-vitro model is truly representative of the in-vivo process, however; they should be regarded as approximations rather than true representations. Nevertheless, in-vitro chemosensitivity assays may play crucial roles in the development of novel therapeutic strategies, especially within clinical trial settings [42]. In the context of our proposal, it may be imperative to perform chemosensitivity assays using different endothelial cell types and incorporating different aspects of angiogenesis, such as endothelial cell proliferation, migration, cell differentiation and processes involving extracellular matrix remodeling. Although the 'holy grail' of cancer treatment is the absolute individualization of a schedule, group profiling using combinations of assays is more likely to be clinically relevant in the near future. Given that these assays are not individualized (patient specific), in the end we envisage that a range of in-vitro metronomic (anti-endothelial) dose values can be established for all agents and exist on an easily accessible database. Their relevance to the clinical situation will, however, need to be defined by the establishment of a clinical efficacy endpoint (see below).

Endpoint (defining the clinical MetD)

The conventional approach to defining a conventional cytotoxic dose is based on the appearance of a set of clinical signs or symptoms that define the DLT. Based on recent insights into what constitute potent clinical angiogenic stimuli, we propose a converse approach.

Fig. 2

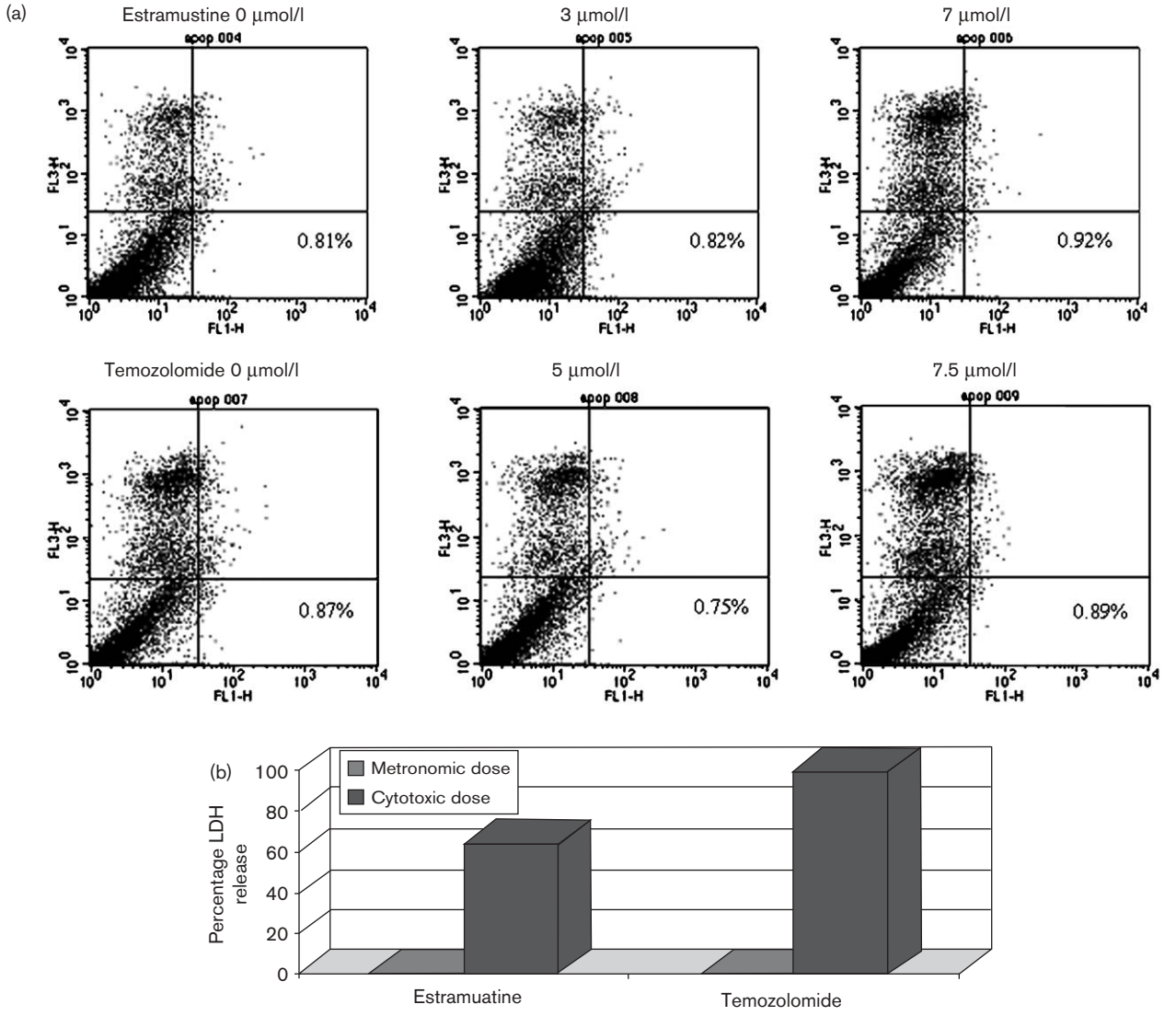


Endothelial cell-specific growth inhibition at low doses following continuous exposure to temozolomide. See legend to Fig. 1 for details. Reproduced with permission from Maraveyas A, Lam T, Hetherington JW, Greenman J. Can a rational design for metronomic chemotherapy dosing be devised? *Br J Cancer* 2005 **92**:1588–1590. Published by Nature Publishing Group

New evidence is emerging to indicate that the process of tumor angiogenesis is not merely restricted to endothelial cells derived from neighboring blood vessels. Highly proliferative, circulating endothelial progenitors (CEPs) derived from the bone marrow may be recruited by tumors to participate in angiogenesis [43]. In a recent study, experiments on angiogenic-defective and tumor-resistant Id-mutant mice showed that tumor angiogenesis and growth could be restored following transplantation of wild-type bone marrow or VEGF-mobilized stem cells [44]. Crucially, the investigators detected donor-derived CEPs throughout the neovessels of tumors, and demonstrated that inhibition of both VEGFR-1 and -2 inhibited tumor growth completely. Release of hematopoietic stem cells including CEPs from the bone marrow into the circulation in response to chemotherapy, cytokine stimulation or irradiation was first documented more than three decades ago (reviewed in [45]). Under such conditions of 'stress', chemokines and cytokines are released from inflammatory cells, and these factors increase stem cell motility, which in turn facilitates their entry into a microenvironment within the bone marrow where they proliferate and differentiate before being mobilized to the circulation [46]. Notably, metronomic dosing schedules of cytotoxic agents can avoid CEP mobilization and reduce CEP viability. Using a model of NOD/SCID mice with lymphoid tumors, Bertolini *et al.* [47] showed that the administration of cyclophosphamide at the MTD (up to 225 mg/kg/cycle) with 21-day breaks resulted in extensive CEP mobilization and rapid acquisition of drug resistance. Conversely, the regular administration of low-dose cyclophosphamide (20 mg/kg/day) either daily (orally) or every 6 days (i.v.) was associated with minimal CEP mobilization and decreased CEP viability, and inhibition of tumor growth was significantly prolonged. Results such as these suggest that the anti-angiogenic effects of metronomic chemotherapy may partly be mediated through the reduction in CEP mobilization and viability, in addition to direct inhibitory effects on the growth, function and survival of activated endothelial cells on tumor vessels.

We would argue that true metronomic protocols should preclude or minimize the development of bone marrow perturbation. However, in optimizing MetD in clinical trials, bone marrow perturbation may occur, especially if in-vitro metronomic drug levels approach the MTD or if the drug has an intrinsically low threshold for inducing marrow perturbation. Under such circumstances, we propose that MetD should be the highest dose that can be delivered in a MetS without causing clinical bone marrow perturbation, assuming that there are no other DLTs that appear before bone marrow perturbation. Monitoring the full blood count is at present a practical

Fig. 3



(a) Standard Annexin-V:FITC/propidium iodide assay for cellular apoptosis using dual-color flow cytometry. Results shown are for controls (no drug) and cells treated with single drug (estramustine 3 and 7 μmol/l; temozolomide 5 and 7.5 μmol/l) after 4 days of incubation. Plots represent Annexin-V:FITC staining (FL1-H) versus propidium iodide (FL3-H), with cells which stain Annexin-V, but exclude propidium iodide (i.e. lower right quadrant), reflecting cells undergoing apoptosis. The percentage values represent the proportion of apoptotic cells in the population. (b) Standard LDH release assay measuring cell lysis in the presence of metronomic (7 μmol/l estramustine and 7.5 μmol/l temozolomide) and cytotoxic doses (100 μmol/l in each case) of drugs. All results are representative of two independent experiments.

approach, but is fraught with weaknesses. Small variations within conventional normal limits of the typically monitored elements (hemoglobin, white count and platelets) can mask a significant amount of precursor fluctuation that can have bearing on the 'metronomic' effect as we have defined it, therefore research into developing assays of bone marrow stem cell activation/circulation (detection of CEPs using cytometry [47] or PCR [48]) or correlative markers such as thrombospondin (TSP)-1 activation [30] are likely to provide more definitive and robust endpoints. However, this work is still at a relatively early stage. Currently the time-course of CEP release is unknown and thus it is unclear how well the concentration in the circulation reflects critical events in the bone marrow. In a recent mouse model study, Shaked *et al.* [49] only provide an insight at a 1-week interval from treatment to assay. We know that in the clinic however, there is a lag time of effects for neutrophils from administration of chemotherapy to nadir to rebound [50]. The timing of these effects is also highly agent dependent. Although this clinical model is for single agents conventionally dosed, unless similar work is undertaken for CEPs/circulating endothelial cells (CECs) there will be little confidence in what a single time-point

result reflects. We do not understand if an initial CEP reduction is a beneficial response to metronomic or an initial response to bone marrow perturbation. Is this reduction the same response as that to bevacizumab [51]? If so, why should adding metronomic enhance the benefit of the anti-VEGF antibody? Further studies at more remote time points are needed.

Not all cytotoxic drugs affect the bone marrow in the same way; therefore, the proposed approach will need to be drug-specific and it is conceivable that this strategy may not apply to all drugs. Nevertheless, it represents an example of how the upper limit of MetD in clinical trials can be determined using a rational approach.

Conclusion

In essence therefore we have postulated a scientific basis for the proposal that a metronomic dosing schedule (MetS) would constitute either an oral agent given daily or protracted i.v. infusion on a continuous basis and the metronomic dose (MetD) is the maximum dose that can be delivered in this schedule without bone marrow perturbation or induction of a pro-angiogenic stimulus. For some agents, metronomic-like dosing already exists, e.g. estramustine, cyclophosphamide and capecitabine. However, this is not the case for the majority. This is not a trivial point, as the nomenclature 'metronomic' has already taken on a confusing quality as we strive to adapt, in the clinical sense, chemotherapy that already exists. The 'metronomic' use of temozolomide with radiotherapy in children causing profound bone marrow toxicity [52] or the development of gemcitabine in a discontinuous (weekly schedule) with similar principles as advocated in our paper (de-escalate the bone marrow toxicity) [53] are but two examples of this. Given that development from scratch is not practical, two possible strategies can be envisaged. Either a dose-escalation study starting, for example, at an arbitrary 10% level of current conventional dose, supported by in-vitro studies of the kind suggested in this review, given daily or a de-escalation study from DLT until no marrow toxicity is clinically apparent and then converting this dose to daily if it is not so already and de-escalating or escalating according to endpoint requirements. We would, however, warn from our experience that even a simple fractionation of a weekly dose to a twice-weekly schedule can lead to profound clinical effects due to the unpredictability of clearance (pharmacokinetics) and tissue effects (pharmacodynamics) of agents and their metabolites [54].

Addressing the issues of empiricism in the development of metronomic dosing would allow the development and design of useful studies looking at surrogate endpoints (e.g. TSP levels in serum, tumor biopsies, etc.) that can be correlated to dosing and schedule. The clinical approach we have advocated is derived from our understanding of events in cell culture and animal models. It must be noted, however, that these models do have inherent weaknesses, especially when extrapolation to a clinical approach is attempted. The cell culture model we have used provides no insight into pharmacokinetics and pharmacodynamics of the studied agents. Animal work can provide some insight into this but is also considerably model dependant, e.g. Shaked *et al.* [31] studying eight different inbred mice found substantial variability in each animal strain of CEP and CEC and their molecular regulators at baseline and in response to pro-angiogenic stimuli and anti-angiogenic drugs (anti-VEGFR-2 antibody). Hence, as with any model, its limitations need to be understood fully.

The gradual, but increasing, shift from the traditional paradigm of cytotoxic chemotherapy based on the MTD towards alternative paradigms is gathering pace and metronomic chemotherapy is an example of a new treatment paradigm. As things stand, it is only a matter of time before the results of clinical trials start providing evidence on the value of this approach in treating cancer. In this short review we believe we have proposed a tangible methodology to develop the multitude of agents that exist in a rational way. We think this is necessary given that extreme empiricism may breed skepticism, which in turn may delay the progress of this exciting field.

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