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Low-dose metronomic chemotherapy: from past experience to new paradigms in the treatment of cancer

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Low-dose metronomic (LDM) chemotherapy represents an emerging concept in the treatment of cancer. Directed against tumor cells and other types of cells, such as endothelial and immune cells, this treatment regimen alters the tumor microenvironment and suppresses innate features which support tumor growth. Ongoing Phase III clinical studies explore various applications of LDM chemotherapy, mostly combined with other anticancer agents, to act as complementary treatments to conventional maximum tolerated dose (MTD) chemotherapy. In this article we summarize preclinical and clinical experience with LDM chemotherapy, emphasizing the potential contribution of this new treatment modality to future paradigms in the systemic treatment of patients with cancer.

In the past decade, the mechanisms of the antitumor activity of low-dose metronomic (LDM) chemotherapy regimens have gradually been elucidated by preclinical and clinical research. Since the year 2000, the definition of metronomic chemotherapy has been changed several times in a manner which does not necessarily reflect the mechanism of action of the drug, but its pace and dose of administration. However, in this mini-review we will not cover the existing arguments and discussions regarding the definition and terminology of LDM regimens [1]. LDM refers to administration of comparatively low doses of a chemotherapeutic or nonchemotherapeutic drug (compared with conventional doses) on a frequent (daily, several times a week, or weekly) or continuous schedule with no extended interruptions. Initially, it was suggested that this type of regimen exerts its effects exclusively by killing the rapidly dividing endothelial cells in tumors, thus preventing angiogenesis [2,3]. Recent evidence, however, implied that additional mechanisms might be involved; these prevent tumor growth by creating a less supportive tumor microenvironment and include selective impairment of subtypes of inflammatory cells, and induction of dormancy in tumor cells [4]. In parallel to ongoing preclinical research, several clinical trials aimed at assessing metronomic chemotherapy, are also under way. These include Phase III studies that combine metronomic chemotherapy regimens with other anticancer drugs such as antiangiogenic drugs or anti-inflammatory agents. In this review, we present updated studies in the field of metronomic chemotherapy both at the bench and in clinical practice.

Preclinical research

When Hanahan and colleagues coined the term metronomic chemotherapy [5], it prompted interest in the scientific community to further evaluate this treatment regimen in both preclinical and clinical studies, aiming to test its activity, and mode of action. In the initial study employing LDM chemotherapy, Lewis lung carcinoma-bearing mice that were resistant to maximum tolerated dose (MTD) cyclophosphamide, were treated with the same drug in once every six days metronomic schedule. Under this LDM treatment regimen, cyclophosphamide showed remarkable decrease in tumor growth [2]. In a back-to-back study, Klement *et al.* demonstrated that continuous administration of vinblastine in an LDM chemotherapy regimen led to extended antitumor activity of neuroblastoma tumors only when the treatment was combined with the antiangiogenic drug DC101, a vascular endothelial growth factor receptor 2 (VEGFR2)-blocking antibody [3]. As a consequence of the increased antiangiogenic treatment

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benefit when both drugs were combined in the Klement study [3]. It was suggested that LDM chemotherapy may have additional mechanisms that contribute to treatment outcome. These possible mechanisms were further investigated and are described below.

The local and systemic antiangiogenic effect of LDM chemotherapy

The antiangiogenic effects of LDM chemotherapy have already been extensively reviewed [4,6]; therefore, here we provide a short summary on the diverse antiangiogenic mechanisms of LDM chemotherapy. First, LDM chemotherapy directly destroys endothelial cells. Several studies have indicated that the prolonged *in vitro* administration of low concentrations of cytotoxic drugs to rapidly dividing endothelial cells, such as human umbilical endothelial cells (HUVECs), increased their apoptosis compared with tumor cells [7]. Second, LDM chemotherapy may also lead to antiangiogenic activation and secretion of angiogenesis inhibitors such as thrombospondin-1 (TSP-1). Tumor-bearing mice who received LDM cyclophosphamide expressed high levels of TSP-1, and exhibited an improved treatment outcome. Such therapy, however, could not induce antitumor activity in TSP-1 null mice, suggesting that the induction of TSP-1 in mice treated with LDM chemotherapy leads to antiangiogenic activity [8]. Clinically, decreased levels of angiogenic factors such as VEGF and platelet-derived growth factor-BB (PDGF-BB) were reported in patients with cancer treated with LDM capecitabine or LDM cyclophosphamide, methotrexate and thalidomide [9,10]. Third, LDM chemotherapy suppresses systemic angiogenesis mediated by circulating bone marrow-derived proangiogenic cells (BMDCs), such as circulating endothelial progenitor cells (CEPs) [11]. Bertolini *et al*. tested the levels of CEPs in lymphoma-bearing mice that underwent LDM versus MTD cyclophosphamide. Although the MTD regimen increased the number of CEPs in peripheral blood, the LDM regimen suppressed their levels during the treatment period [12]. Maximal suppression in CEP levels following LDM chemotherapy was found to correlate with the maximum antiangiogenic effect that led to the greatest antitumor activity of the therapy [11]. Lastly, it seems that the antiangiogenic activity of LDM regimens is dependent in part on the drug or the combination of drugs used in this treatment regimen.

LDM chemotherapy and the immune system

A growing body of evidence demonstrates that LDM chemotherapy of drugs such as temozolomide or cyclophosphamide, can deplete T regulatory cells, hence, can increase the antitumor activity of additional drugs used concomitantly with the LDM regimen, such as antiangiogenic drugs and anti-inflammatory agents [13,14]. For example, daily oral administration of the topoisomerase inhibitor topotecan, can stimulate the expression of major histocompatibility complex (MHC) class I in breast cancer by increasing the levels of several factors known to induce antigen presenting molecules such as interferon-β (IFN-β). By doing so, such therapy triggers the immune response through T cell cytotoxicity to enhance antitumor activity [15]. Doloff and Waxman reported that the induction of antitumor immune response following LDM cyclophosphamide resulted in the recruitment of natural killer cells, macrophages and dendritic cells into the tumor; this may explain the improved treatment outcome [16]. They further demonstrated that such treatment in Nonobese diabetic/severe combined immune deficient (NOD/SCID) mice which lack many components of the immune system, resulted in treatment failure in some models of cancer [16]. Comparable results were found in L-TACB lymphoma tumors implanted in nude mice that received LDM cyclophosphamide. Although tumor regression was not observed in the treated mice, tumor growth doubling times were significantly higher in the treated group implying other antitumor activity mechanisms independent of immune response to LDM chemotherapy [17]. Antimicrotubule agents administered in nontoxic low doses (e.g. vincristine or vinblastine) can enhance the activity and maturation of dentritic cells [18]. Interestingly, to explain the benefit of combining LDM chemotherapy with an antiangiogenic drug, it has been shown that blocking the VEGF receptor can improve innate immunity and result in overall tumor regression [16]. A decrease in T regulatory cell levels following LDM cyclophosphamide was also found in metastatic breast cancer patients only at the initial phase of treatment, after which the cells recovered, suggesting a transient change in T regulatory cells [19]. Overall, it seems that the suppression of T regulatory cells by LDM cyclophosphamide facilitates a restoration of innate immunity in addition to T cell and natural killer cell activity in tumors.

A combination of LDM cyclophosphamide and oncolytic virus gene therapy, can suppress or deplete several immune cell types which act against the injected virus [20]. Such combinations were found to be efficacious in several preclinical models of malignancies, such as B16 melanoma [20], ovarian carcinoma [21], glioblastoma [22], pancreatic cancer [23], among others. Administration of daily low doses of etoposide together with oncolytic herpes virus in a mouse model of glioblastoma-relapsed tumors showed a remarkable increase in survival and an induction of apoptotic tumor cells [22]. Interestingly, the enhanced antitumor activity was related to the impact of the combined therapy on cancer stem cells (CSCs) (as we will discuss below). Thus, the purpose of LDM chemotherapy is not necessarily to inhibit the immune response against the virus, but rather to directly and specifically affect relapsed tumor cells [22]. Similarly, in recurrent ovarian cancer, treatment with a combination of LDM paclitaxel and oncolytic virus led to increased efficacy. Here, paclitaxel, a microtubule-stabilizing agent, promoted morphological changes in the replicated tumor cells and induced tumor cell apoptosis after the cells were infected with the virus, improving the combination therapy [21]. Thus, combinations of LDM chemotherapy and other drugs were aimed at inducing innate immunity against tumor cells, and reducing immune response against oncolytic virus therapy.

Preventing host acute effects by metronomic chemotherapy

Recent studies have indicated that MTD chemotherapy can result in rapid host effects which may not be seen following LDM therapy [12,24]. For example, an acute elevation of various plasma factors such as granulocyte colony-stimulating factor (G-CSF) and stromal-derived factor-1 (SDF-1) has been shown in response to MTD chemotherapy [25]. Such factors were found in both tumor-bearing and nontumor-bearing mice suggesting they are host-driven factors. Other studies have indicated that these host responses to acute therapy may induce angiogenesis and metastases [26,27]. Elevated levels of host proangiogenic factors in

response to therapy have also been reported following the use of vascular disrupting agents (VDAs) [28], and some targeted anti-angiogenic drugs, such as sunitinib malate [29]. Although there is no evidence yet that such host effects may also take place following LDM chemotherapy, there are some clues that an LDM regimen may, in fact, inhibit the host effects that occur following acute therapy. For example, Daenen *et al.* have demonstrated that the combination of a VDA with continuous administration of LDM cyclophosphamide resulted in decreased tumor cell regrowth compared with that expected following VDA therapy [30]. In addition, studies have shown that the administration of an LDM regimen following an acute dose of chemotherapy markedly improved the treatment outcome of pancreatic, breast and prostate cancers in addition to erythroleukemia [31,32]. In some of these aggressive models, combined therapy was used in a 'chemo-switch' regimen in which one cycle of MTD regimen was followed by LDM chemotherapy combined with targeted agents [31].

Although LDM regimens themselves have antiangiogenic effects, a remarkable synergistic effect was observed when LDM regimens were combined with an antiangiogenic drug [4,6]. Administration of LDM topotecan with pazopanib, a potent selective tyrosine kinase inhibitor [33], has shown significant improvement in overall survival of mice bearing metastatic ovarian cancer. LDM topotecan exhibited excellent antitumor activity, which was further enhanced by concurrent pazopanib therapy [34,35]. How can an acute dose of chemotherapy (or targeted drug) followed by maintenance LDM chemotherapy induce these dramatic antitumor effects? Possible mechanisms to explain the enhanced treatment efficacy of the combined therapy could be the reduction in systemic involvement of BMDCs that are usually mobilized in response to acute therapy [32], or the inhibition of several circulating proangiogenic factors induced by the targeted drugs using the LDM chemotherapy [34,35]. Taken as a whole, 'blunting' of the protumorigenic activities, which may sometimes occur in the reacting host following an acute therapy, may provide a reasonable basis to combine MTD with LDM chemotherapy even when the same drug is being used in both regimens.

LDM therapy may disrupt the CSCs niche

An emerging concept in cancer biology is related to the hypothesis that a subpopulation of cells in tumors acts as stem cells and, therefore, can initiate tumor growth. As such, these cells were termed CSCs or tumor initiating cells (TICs) [36]. CSCs display important properties such as the ability to initiate tumors and to drive cell proliferation, to differentiate into multilineage 'mature' tumor cells, and to maintain a self-renewal capacity [36]. They also contain DNA repair systems which distinguish them from other tumor cells [37]. Recent studies have demonstrated that CSCs are resistant to many conventional anticancer drugs, therefore efforts to search for treatments that would eliminate CSCs are being undertaken. Mounting evidence has suggested that CSCs remain in close proximity to the tumor vasculature [38]. The disruption of the VEGF–neuropilin axis resulted in reduced CSCs properties, suggesting that the paracrine secretion of VEGF-A, in addition to neuropilin, maintain these properties [39]. Antiangiogenic therapy, therefore, could be a possible treatment strategy to eradicate CSCs; indeed, several preclinical studies indicated that anti-VEGF therapy may reduce the number of CSCs in treated tumors. Because LDM chemotherapy has antiangiogenic properties, it should be evaluated as a possible treatment to target CSCs; however, there is limited evidence for the anti-CSCs action of LDM chemotherapy. Treatment of C6 rat glioma-bearing mice with LDM cyclophosphamide alone or in combination with an antiangiogenic drug led to a reduced number of sphere-forming tumor cells, suggesting that they are CSCs [40]; however, such anti-CSCs effects were also observed when MTD chemotherapy was combined with an antiangiogenic drug [40]. In a hepatocellular carcinoma model, the combination of LDM cyclophosphamide with an antiangiogenic drug induced CSC dormancy. Once LDM therapy was terminated, tumor regrowth was observed, suggesting that LDM chemotherapy promoted tumor dormancy of the residual disease [41]. Furthermore, glioblastoma recurrence following treatment with LDM etoposide and oncolytic herpes virus resulted in extended survival due to increased number of apoptotic CSCs, suggesting that LDM chemotherapy may interfere with the growth of CSCs in recurred tumors [22]. The developing research in the CSCs field suggests that LDM chemotherapy may inhibit or reduce CSCs thus promoting tumor dormancy, and therefore can be used as a maintenance therapy to avoid the growth of relapsed tumors.

Clinical research

In this section we provide an update on clinical research conducted with LDM chemotherapy. A comprehensive literature search of the MEDLINE database was performed using the key words 'metronomic chemotherapy', 'low dose chemotherapy' and 'cancer'. Only Phase II clinical studies or retrospective reports with equivalent numbers of patients were chosen, and then grouped and reviewed by disease identity. In addition, a search focused on ongoing Phase III clinical studies was conducted in the clinicaltrials.gov (http://clinicaltrials.gov/) database.

Past clinical experience

It is recognized that the administration of chemotherapy drugs in low doses, continuously, has been successfully practiced as a maintenance treatment in patients with leukemia long before the concept of metronomic chemotherapy was actually introduced [42]. Past clinical experience with LDM regimens in solid tumors has been limited by their empirical nature regarding the identity of the drugs in addition to their doses [43]. Furthermore, many of the trials suffered from insufficient numbers of patients and patient heterogeneity. The relevant literature reveals that oncologists initially were attracted by the friendly characteristics of LDM regimens: easy administration, mainly orally and on an ambulatory basis, with low-cost drugs and a low-degree of toxicity. It made LDM regimens most appealing for elderly and frail patients, such as those with prostate or breast cancer, and for others as well. Consequently, oncologists have tried to administer LDM regimens in 'rescue' conditions that were usually limited to heavily pretreated patients with a wide range of cancers, including breast [44] (Tables 1 and 2), prostate [45], ovary [46,47], colon [48,49], lung [50], stomach [51], liver [52], adrenocortical [53], cutaneous angiosarcoma [54], glioblastomas [55] and pediatric cancers [56,57]. The initial results demonstrated that even in this unfavorable setup, different response rates and clinical benefits could be achieved [44], including a significant extension of event free survival in patients with high-risk anaplastic large-cell

TABLE 1

Clinical studies using only chemotherapeutic agents in LDM regimens for breast carcinoma

The drug(s) used	Study type	No. of patients	No. of patients ER+	Number of previous therapy lines	%OR + %NC = %CBR	Median TTP (months)	Toxicity: grade ≥III (% of patients)	Comments	Refs
CTX + MTX	Prospective	63	31/63	1 line: 32/63 2 lines: 11/63 3 lines: 9/63	19% + 12.7% = 31.7%	2.8	2^{NE}; 2^{ANE}; 15^{TRA}	PD at entry: 51/63	[44]
A = CTX	Retrospective	22	19/22	1 line: 2/22 2 lines: 20/22	14% + 41% = 51%	3.8	5^{NE}		[68]
B = CTX + MTX		39	31/39	1 line: 2/39 3 lines: 7/39	20% + 31% = 51%	4.2	3^{NE}; 3^{TH}		
5′-DFUR + CTX	Prospective	64	21/64	None: 33/64 1 line: 16/64 ≥2 lines: 15/64	29.7% + 17% = 46.7%	NR	1.5^{NE}	Only semi-metronomic[a]	[69]
CTX + capecitabine	Prospective	68	37/68	None: 15/68 1 line: 30/68 ≥2 lines: 23/68	33.3% + 19.7% = 53%	5.2	4.4^{NE}; 1.5^{TRO}; 7.5^{ANE}; 1.5^{NA}; 1.5^{ST}; 1.5^{DI}; 1.5^{TRA}; 4.4^{HF}	Only semi-metronomic[a]	[70]
Capecitabine	Prospective	58	47/58	1 line: 33/60 2 lines: 12/60 ≥3 lines: 15/60	24% + 38% = 62%	7	5^{HF}	13/58 patients were resistant to prior MTD capecitabine	[58]
Vinorelbine	Prospective	34	21/34	None	38% + 32% = 70%	7.7	9^{NE}; 9^{ANE}; 3^{TRO}; 6^{FI}; 3^{DI}; 3^{NA}; 3^{vo}; 3^{ST}	All elderly pats. = age 70–84	[61]

Abbreviations: ANE: anemia; ANO: anorexia; CBR: clinical benefit rate; CTX: cyclophosphamide; DIA: diarrhea; ER+: estrogen receptor positive; HF: hand-foot syndrome; MTX: methotrexate; NA: nausea; NR: not reported; NC: no change ≥ six months; NE: neutropenia; OR: objective response; PD: progressive disease; ST: stomatitis; TTP: time to progression; TRA: transaminitis; TRO: trombopenia; VO: vomiting.

[a] Semi-metronomic regimen, unlike 'true' LDM regimen, represents the administration of drug(s) in cycles, which include metronomic-like repetitive doses, yet reaching a cumulative dose in the MTD range, hence imposing drug-free break periods.

lymphoma by prolonged weekly treatment with vinblastine [42]. Interestingly, antitumor activity could be obtained even if the drug was already inactive in the same patients following previous conventional MTD treatments [58]. Therefore, it seems that the basic principles of activity of LDM chemotherapy as elucidated in animal studies [2,3,59] may in fact be reproducible in the clinic. Consequently, clinical investigators are currently expanding their research with LDM chemotherapy both by studying the effectiveness of the therapy when it is combined with biological new antiangiogenic drugs, and by testing various indications for the introduction of LDM chemotherapy among the consecutive lines of systemic treatments for patients with cancer. These new paradigms include 'rescue' treatment, 'maintenance' following response achieved by MTD chemotherapy, and even 'consolidation' of the disease-free condition in patients at high risk of relapse following neo and/or adjuvant chemotherapy. These three types of clinical indications are usually planned for patients who have exhausted all other MTD treatments, enabling LDM chemotherapy to have unique roles by virtue of its low toxicity profile and its mostly oral and ambulatory route of administration. The accumulated clinical experience with LDM chemotherapy in various types of cancer has already been reviewed by others [4,45]. We therefore directed our efforts in reviewing the relevant situation in only four representative diseases: breast, prostate, ovary and colorectal cancers (CRCs) in which we commented on the characteristics of the clinical experience and the conclusions drawn.

Breast carcinoma

The seminal prospective clinical study with LDM chemotherapy in patients with metastatic breast carcinoma was published in 2002 by Colleoni *et al.* [44] who administered cyclophosphamide (50 mg) once daily and methotrexate (2.5 mg) twice daily. As shown in Tables 1 and 2, this Phase II study and others were limited by a small number of participants and suffered from heterogeneity of patient populations and disease characteristics. Nevertheless, they supported several conclusions: first, that LDM chemotherapy treatment deserves to be further studied clinically toward wider applications based on substantial and impressive rates of clinical benefit lasting beyond six months; second, the activity of LDM chemotherapy is possible even in heavily pretreated patients with metastatic disease who no longer respond to conventional therapy, sometimes even when similar drugs have been previously used under an MTD regimen in the same patients [58]; third, the potential effect of LDM chemotherapy is not limited to one specific drug regimen; fourth, LDM chemotherapy can potentiate the activity of certain biologically targeted drugs, sometimes even in patients who have previously been treated with the same targeted agents under a different regimen [60]; and fifth, the grade of toxicity related to LDM chemotherapy is usually low, and limited to just a few percentages of cases that are grade 3 or above, thus enabling its convenient administration even in the elderly [61] and/or in heavily pretreated patients. These conclusions support further clinical research, which would have to be conducted in randomized studies and focused on well-defined patient populations and disease subtypes.

Prostate carcinoma

Patients with castration-resistant prostate cancer (CRPC) have been sporadically and empirically treated by different metronomic chemotherapy regimens. A literature review on oral metronomic

TABLE 2

Clinical studies combining chemotherapeutic and other agents in LDM regimens for breast carcinoma

The drug(s) used	Study type	No. of patients	No. of patients ER+	Number of previous therapy lines	%OR + %NC = %CBR	Median TTP (months)	Toxicity: grade ≥III (% of patients)	Comments	Refs
CTX + MTX + dalteparin + prednison	Prospective	41	29/41 Unknown	None: 16/41 1 line: 10/41 2 lines: 5/41 ≥3lines: 10/41	17% + 7% = 24%	2.5	$2.5^{NA/VO}$; 2.5^{FA}; 27.5^{TRA}		[71]
A = letrozole	Prospective/ randomized	57	ALL	None	71.9% + 21% = 92.9%	NR		Preoperative treatment	[72]
B = CTX + letrozole		57			87.7% + 7.1% = 94.8%		0.5^{TRO}; 0.5^{CYST}		
A = CTX + MTX	Prospective/ randomized	90	50/90	None: 37/90 1 line: 27/90 ≥2 lines: 26/90	20.9% + 20.6% = 41.5%	3.8	5^{NE}; 1^{TRO}; 2^{ANE}; 10^{TRA}; $1\%^{skin\ toxicity}$	PD at entry: A = 61/90	[10]
B = CTX + MTX + thalidomide		88	49/88	None: 33/88 1 line: 35/88 ≥2 lines: 20/88	11.8% + 29.7% = 41.5%	4.1	1^{DIA}; $1^{NA/VO}$ 1^{NE}; 2^{TRO}; 1^{ANE}; 13^{TRA}; $1^{skin\ toxicity}$	B = 62/88	
CTX + MTX + trastuzumab	Prospective	22	7/22	None: 1/22 1 line: 8/22 2 lines: 8/22 3 lines: 5/22	18% + 28% = 46%	6	2^{TRA}	All positive to HER-2 and pretreated with trastuzumab ± MTD	[60]
CTX + capecitabine + bevacizumab	Prospective	46	35/46	None: 27/46 1 line: 11/46 ≥2 lines: 8/46	48% + 17% = 65%	10	4.3^{NE}; 4.3^{TRA}; 17.4^{HYPERT}		[73]
CTX + capecitabine + bevacizumab + erlotinib	Prospective	24	10/24	None: 21/24	62% + 13% = 75%	8.2	$4^{FI}/4^{DIA}/4^{SAE}$ 8^{HYPERT} $4^{THROMBOSIS}$	1. Low ER expression (<50%) in10/24 patients 2. TNBC in 14/24 patients	[74]

Abbreviations: ANE: anemia; CBR: clinical benefit rate; CTX: cyclophosphamide; DIA: diarrhea; ER+: estrogen receptor positive; FA: fatigue; FI: febrile infection; HY: hypertension; MTX: methotrexate; NA: nausea; NC: no change ≥ six months; NE: neutropenia; NR: not reported; OR: objective response; PD: progressive disease; SAE: serious adverse event; TNBC: triple negative breast cancer; TRA: transaminitis; TRO: trombopenia; TTP: time to progression; VO: vomiting.

cyclophosphamide in this population, recently published by Nelius *et al.* (based on 12 studies, and a total of 353 patients), illustrated the existing interest for using LDM chemotherapy regimens for this population, but also the lack of systematic research in this area [45]. The authors concluded that oral cyclophosphamide is active in the treatment of CRPC even in patients previously treated with MTD docetaxel [62]. This conclusion has been supported by two additional recent Phase II studies on patients with CRPC progressing after docetaxel-based chemotherapy [63,64]. However, the variability of the reviewed LDM regimens and of the corresponding degrees of response in small trials reflects yet unsettled questions that require further clinical research.

Ovary carcinoma

Burger's study [65] showed that ovarian carcinoma can respond to bevacizumab even when administered as a single drug. Therefore, the study by Garcia *et al.* [46] which combined LDM cyclophosphamide with bevacizumab and revealed a similar rate of response, raises a question as to the practical contribution of LDM cyclophosphamide in this setup. Clinical data are missing for defining the role of LDM chemotherapy in ovarian cancer, at least in the case of cyclophosphamide and bevacizumab. In preclinical studies, mice bearing metastatic ovarian cancer treated with pazopanib and LDM topotecan have shown increased survival [34,35]. It would be of interest to test this treatment combination in clinical settings.

Colorectal carcinoma

LDM chemotherapy with daily UFT (a 5-fluorouracil pro-drug) and irinotecan was investigated in patients with CRC in the adjuvant setting in view of high risk for recurrence. A total of 24 patients in stage IIIb and 25 in stage IV with distant metastases following curatively resection operations were enrolled [48]. Results were favorable as reflected from a five-year overall survival of 73% for stage IIIb and 62% for stage IV resected. In another study LDM UFT was administered to patients with metastatic CRC as a maintenance treatment following induction of response. Efficacy and high feasibility were also reported in this trial [49]. Another study on metronomic chemotherapy reports on 38 patients with advanced colorectal or other gastrointestinal malignancies. This study was based on UFT and cyclophosphamide combined with celecoxib, also including an initiating bolus of cyclophosphamide. Disease stabilization was achieved in 45% of the patients while pharmacokinetic studies showed a significant correlation between higher 5FU AUC and C_{max} values and clinical benefit, as reflected by disease stabilization and prolonged progression-free survival/overall survival (PFS/OS)[66]. These studies warrant further clinical investigation of LDM chemotherapy in CRC as a complementary treatment to MTD regimens.

Toward the future: ongoing Phase III trials

Several randomized Phase III clinical trials with LDM chemotherapy are registered under the NIH clinical trials database (http://clinicaltrials.gov/). These offer new paradigms for the comprehensive treatment of patients with cancer with incorporation of LDM chemotherapy as follows: first, in patients with advanced and/or incurable disease for whom a currently recommended regimen bears potential limiting toxicity, LDM chemotherapy is evaluated as an alternative 'first line treatment' with reduced toxicity and noninferior effectiveness. This possibility is being studied in women with HER-2 negative, locally advanced or metastatic breast cancer, comparing bevacizumab plus paclitaxel to bevacizumab plus metronomic cyclophosphamide and capecitabine (NCT01131195); second, there are ongoing studies with LDM chemotherapy as a 'consolidation therapy', following either neoadjuvant (NCT00925652) or adjuvant (NCT01112826) MTD chemotherapy, aimed at extending the disease-free period. In both studies, LDM chemotherapy consists of capecitabine combined with bevacizumab as compared with observation alone. Third, the use of LDM chemotherapy as 'maintenance' therapy is being evaluated in patients with colorectal carcinoma as a substitute to MTD chemotherapy. The CAIRO3 study (NCT00442637) evaluates the use of capecitabine and bevacizumab versus observation, after induction by MTD chemotherapy in combination with bevacizumab. In a second study (NCT01229813) patients with mutated KRAS who are in response following 18 weeks of MTD chemotherapy are randomized to either bevacizumab or LDM chemotherapy with capecitabine. Each of these studies may provide support for the implementation of LDM chemotherapy with an additional practical role in the treatment of patients with cancer, and not only as a 'rescue' treatment following exhaustion of available lines of conventional therapies.

Concluding remarks

The recent experience with LDM chemotherapy and the accumulating data from ongoing preclinical research may suggest several conclusions and implications. First, extensive research is still necessary to uncover the mechanisms of action of LDM chemotherapy. As illustrated in Fig. 1, we have provided a variety of mechanisms which can currently explain the antitumor activity of LDM chemotherapy, but this figure is far from completion; second, both preclinical studies and clinical experience seem to establish LDM chemotherapy as a new treatment modality in oncology; third, LDM chemotherapy could have a role in oncology as an additional and/or a supplementary modality to conventional MTD chemotherapy, by virtue of both its proven antitumor effects and its lower toxicity profile; fourth, the ongoing Phase III studies may pave the way for a more established clinical practice of this regimen under certain conditions of cancer treatment. These conditions could include 'palliation' in patients with advanced disease, or 'consolidation' of disease-free period following neo and/or adjuvant treatment in patients at high risk for recurrence, or 'maintenance' as a temporary substitute for MTD chemotherapy while offering a less toxic alternative and better quality of life; fifth, new Phase II studies are required to recapitulate the newly emerging preclinical findings related to LDM chemotherapy. For example, there are reports on more effective newly emerging drug and/or combinations of LDM chemotherapy, which could improve clinical treatment, for example, topotecan as an inhibitor of hypoxia-inducible factor 1 (HIF-1) in ovarian cancer [35], or propanolol in combination with paclitaxel for breast cancer as an example of drug repositioning in combination with metronomic chemotherapy [67]; and sixth, different LDM regimens might be active in the same subpopulation of patients bearing a certain subtype of disease. This would indicate that there is a clear need for predictive biomarkers to choose the treatment of choice. These

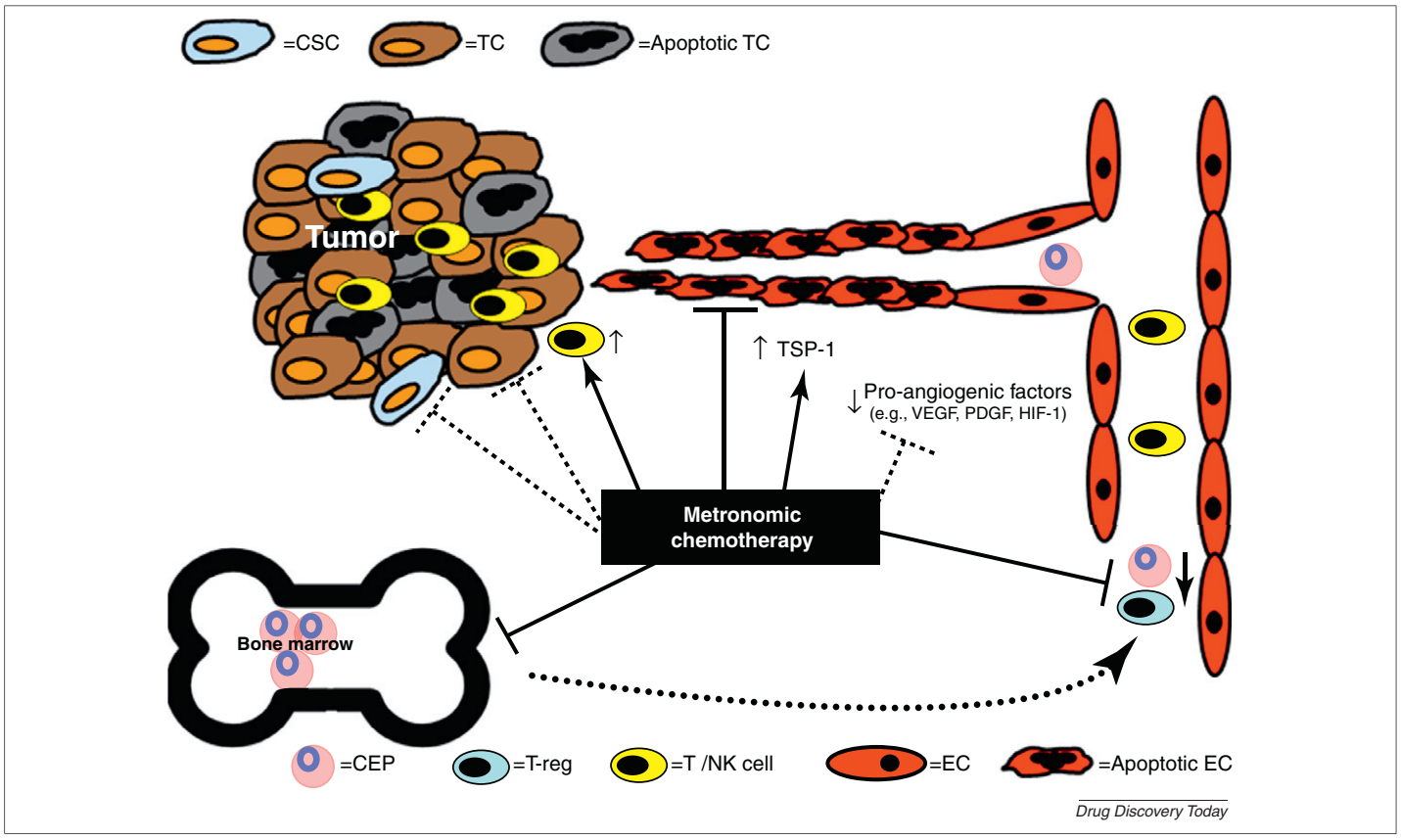

FIGURE 1

Possible mechanisms of action of LDM chemotherapy. Metronomic chemotherapy may act to inhibit tumor growth through various mechanisms: (i) direct tumor cell death; (ii) eradication and disruption of cancer stem cells (CSCs); (iii) direct endothelial cell death through upregulation of antiangiogenic factors [e.g. thrombospondin-1 (TSP-1)] and downregulation of proangiogenic factors [e.g. vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) or hypoxia-inducible factor-1 (HIF-1)]; (iv) blocking of mobilization and decrease of viability of bone marrow-derived circulating endothelial progenitor cells (CEPs), known to contribute to neo-angiogenesis; and (v) suppression of T regulatory cells, and therefore induction of the activity of T cytotoxic cells and natural killer cells. The illustration was adopted from Shaked *et al.* [11] with permission from the journal, and with some modifications. *Abbreviations*: CEP: circulating endothelial precursor cell; CSC: cancer stem cell; EC: endothelial cell; TC: tumor cell; T/NK cell: T cytotoxic or natural killer cell; T-reg: T regulatory cell.

studies emphasize the importance and need for better-planned clinical studies for evaluation of LDM chemotherapy rather than the current prevalent empirical approach. Nevertheless, it is expected that eventually LDM regimens will become a part of the comprehensive management of cancer.

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