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Mini-review

# The potential clinical promise of 'multimodality' metronomic chemotherapy revealed by preclinical studies of metastatic disease

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ABSTRACT

We present a rationale for further clinical development and assessment of metronomic chemotherapy on the basis of unexpected results obtained in translational mouse models of cancer involving treatment of advanced metastatic disease. Historically, mouse cancer therapy models have been dominated by treating established primary tumors or early stage low volume microscopic disease. Treatment of primary tumors is also almost always the case when using genetically engineered mouse models (GEMMS) of cancer or patient-derived xenografts (PDXs). Studies using such models, and others including transplanted cell lines, often yield highly encouraging results which are seldom recapitulated in the clinic, especially when assessed in randomized phase III clinical trials. While there are likely many different reasons for this discrepancy, one is likely the failure to recapitulate treatment of advanced visceral metastatic disease in mice. With this gap in mind, we have developed a number of models of metastatic human tumor xenografts (and more recently, of mouse tumors in syngeneic immunocompetent mice). A pattern of response we have observed with various targeted agents, e.g. VEGF pathway targeting antiangiogenic drugs or trastuzumab, is efficacy when treating primary tumors in contrast to a complete or severely reduced lack of such efficacy when treating advanced metastatic disease. Interestingly, an exception to this pattern has been observed using various continuous low-dose metronomic chemotherapy regimens, where counterintuitively, superior responses are observed in the metastatic setting, as well as superiority or equivalence of metronomic chemotherapy over standard maximum tolerated dose (MTD) chemotherapy, with lesser toxicity. The basis for these encouraging results may be related to the multiple mechanisms responsible for the anti-tumor effects and longer duration of metronomic chemotherapy regimens made possible by lesser toxicity. These include antiangiogenesis, stimulation of the immune system, stromal cell targeting in tumors, and possibly direct tumor cell targeting, including of cancer stem cells (CSCs). In addition, metronomic chemotherapy regimens minimize or even eliminate the problem of chemotherapy-induced host responses that may actually secondarily promote tumor growth and malignancy after causing an initial and beneficial anti-tumor response. We suggest that future preclinical studies of metronomic chemotherapy should be concentrated in the following areas: i) further comparative assessment of anti-tumor efficacy in primary vs metastatic treatment settings; ii) rigorous comparative assessment of conventional MTD chemotherapy vs metronomic chemotherapy using the same agent; iii) assessment of potential predictive biomarkers for metronomic chemotherapy, and methods to determine optimal biologic dose and schedule; and iv) a further detailed assessment of the potential of different chemotherapy drugs administered using MTD or metronomic regimens on stimulating or suppressing components of the innate or adaptive immune systems.



## Introduction

*Developing translational therapy models of metastatic cancer*

For decades a recurring problem in cancer research has been the poor reliability of preclinical therapy studies undertaken in mice to predict subsequent clinical activity, at least at the randomized phase III clinical trial level [1,2]. A common problem is 'over-prediction', i.e., encouraging, even spectacular therapy outcomes in mice which turn out to be false positives, as it is not uncommon for this to be followed later by complete failure in phase III clinical trials. Indeed, over 60% of hugely expensive randomized phase III trials in oncology fail despite earlier phase II and preclinical results which looked positive [3,4]. As a result, there has been a significant effort over the last 20 years to try and improve the predictive power

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of preclinical mouse therapy models (and the design of phase II trials) and the two most significant preclinical initiatives in this regard have been the use of either spontaneous tumors arising in genetically engineered mouse models (GEMMs), or patient derived xenografts (PDXs) – as opposed to the use of tumors generated by transplantation of tumor cells, usually from established cultured cell lines. It is still not clear how much the clinical predictive potential the GEMM or PDX approaches have improved compared to models involving direct transplantation of tumor cells.

Around 2004 a translational research program was initiated evaluating a different approach for improving the predictive potential of preclinical mouse models, namely, to try and recapitulate in mice treatment of late stage (advanced) metastatic disease [2,5] and then later of early stage micro-metastatic disease [6–8]. The rationale for doing so is that the vast majority of preclinical mouse therapy studies involve treatment of established primary tumors, and this is also true for GEMMs and PDXs. The two latter approaches are generally associated with a very low incidence of spontaneous visceral metastatic disease [9], although there are some exceptions, at least with GEMMs [10–13]. Metastatic disease, especially when advanced in nature, is a far more difficult clinical circumstance to successfully treat. In such cases, most treatments are palliative in nature, whereas in the neoadjuvant or adjuvant circumstances, treatments can be potentially curative. We have developed a number of models beginning with human breast cancer in immune suppressed mice [14], then human colorectal [15], renal [16], and ovarian carcinoma [17] as well as malignant melanoma [18] and locally advanced orthotopic hepatocellular carcinoma (HCC) [19].

The methodology for developing these aforementioned models, in most cases, has involved orthotopic transplantation and serial selection of spontaneous metastases in vivo. For example, using the MDA-MB-231 triple negative human breast cancer cell line, cells are injected into the mammary fat pads of female severe combined immunodeficient (SCID) mice. Orthotopic transplantation is a known method of promoting distant spontaneous metastatic spread [2]. However, detection of such metastases may necessitate surgical resection (mastectomy) of the primary tumor to allow sufficient time for overt metastases to develop in such sites as the lungs, liver or lymph nodes. We found that such distant overt metastases took between 4 and 6 months to develop when detected by gross inspection [14]. These were mostly found in the lungs, and were pooled to establish a subline called LM1. The LM1 cells were then injected into the mammary fat pads and the procedure repeated in a second group of SCID mice [14]. This subsequent in vivo selection resulted in accelerated and more robust metastatic disease not only in the lungs but in some mice, the liver and lymph nodes [14] after surgical resection of the primary tumor (Fig. 1A). A single lung metastasis was selected and a cell line established from it, called LM2-4. This line was then used for experimental therapy studies usually involving orthotopic transplantation, surgical resection, and then initiating therapy approximately one month later when mice develop distant metastatic disease in a heterogeneous fashion (Fig. 1B).

Importantly, the metastatic variant subline cells can be readily tagged with an imageable biomarker, eg. luciferase, so that whole body bioluminescent imaging can be undertaken to monitor the progression and therapeutic response of metastatic disease [6] (something that cannot be done easily, if at all, with PDX tissue models). An example is shown in Fig. 1B where mice with orthotopic primary tumors are imaged prior to and then 5 or 30 days after surgical resection of the primary tumors. This illustrates how initiating therapy within a few days of primary tumor resection would constitute a version of adjuvant therapy of early stage micrometastatic disease, whereas waiting a month before initiating therapy would constitute treatment of overt metastatic disease. Therapeutic outcomes in these models can be very different depending on what stage of disease progression therapy is initiated, as discussed below.

## Results – using antiangiogenic drugs

An obvious question is whether these metastatic models better reflect clinical biology and therapeutic outcomes compared to the conventional approach of utilizing established primary tumors. Three examples follow which suggest this may indeed be the case. First, we recapitulated an important clinical therapeutic outcome of growing importance, namely, the emergence of spontaneous brain metastases in mice that had no evidence of such metastases when a successful treatment was initiated [18]. Presumably, as the result of extending the survival times of mice with systemic disease allowed what were asymptomatic cryptic (microscopic) brain metastases more time to develop into overt lesions [20,21]. This brain 'sanctuary' phenomenon is a disheartening observation in women metastatic HER2+ cancer who experience prolonged survival with trastuzumab-based therapy [21]. Second, we undertook a comparative analysis of the therapeutic effects of antiangiogenic drugs in the common circumstance of treating established primary tumors, *vs* the much less common preclinical circumstance of treating established visceral metastatic disease. An example of this is shown in Fig. 2. We found that oral antiangiogenic TKIs such as sunitinib or pazopanib as well as an antibody to mouse VEGFR-2 (called DC101) all caused anti-tumor efficacy when treating orthotopic primary tumors (Fig. 2A, which shows the sunitinib results) [22]. In contrast, no such efficacy was detected when treating overt metastatic disease, which was mainly detected in the lungs (Fig. 2A). Moreover, when sunitinib was combined with paclitaxel chemotherapy, no efficacy was noted in the metastatic treatment setting compared to other treatment groups (Fig. 2B). Addition of paclitaxel chemotherapy to sunitinib did not change the outcome in the metastatic setting. In contrast the combination of DC101 plus paclitaxel did cause a survival benefit in line with the results of a prior phase III clinical trial called E2100 in metastatic breast cancer patients [23]. A lack of benefit to sunitinib treatment of metastatic disease has been recently confirmed in two other metastatic models, using the 4T1 basal-like mouse breast and mouse C26 colon cancer cell lines [24]. Third, we also reported results that foreshadowed the subsequent failure of adjuvant antiangiogenic therapy for the treatment of early stage micrometastatic disease [6], including breast cancer [25]. When the primary LM2-4 breast cancer was resected and adjuvant treatment initiated immediately, the therapeutic outcome was actually worse in the treated mice compared to vehicle control [6]. These results had 'cautionary implications' for the clinical assessment of antiangiogenic drugs in the adjuvant setting [6]. In the following years, three breast cancer randomized adjuvant trials of bevacizumab plus chemotherapy have been undertaken and all failed to meet their primary endpoint [7,25]. Although none showed a worse outcome, it is notable that these trials all involved bevacizumab plus chemotherapy followed by maintenance bevacizumab. It is thus possible that the combination with chemotherapy in patients prevented any potential pro-malignancy effect induced by the antiangiogenic drug. Indeed, we recently published some evidence in support of this hypothesis [26]. In this same paper we also reported that a sequenced combination of neoadjuvant therapy, surgical resection, followed by adjuvant antiangiogenic based therapy (i.e., with chemotherapy) could bring about an OS effect and this too recapitulated a secondary analysis of a recent neoadjuvant-adjuvant phase III



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