Cancer Therapy: Preclinical

Effective Treatment of Advanced Human Melanoma Metastasis in Immunodeficient Mice Using Combination Metronomic Chemotherapy Regimens

William Cruz-Munoz, Shan Man, and Robert S. Kerbel

Abstract

Purpose: The development of effective therapeutic approaches for treatment of metastatic melanoma remains an immense challenge. Present therapies offer minimal benefit. Although dacarbazine chemotherapy remains the standard therapy, it mediates only low response rates, usually of short duration, even when combined with other chemotherapeutic agents. Thus, new therapeutic strategies are urgently needed.

Experimental Design: Using a newly developed preclinical model, we evaluated the efficacy of various doublet metronomic combination chemotherapy against established advanced melanoma metastasis and compared these with the standard maximum tolerated dose dacarbazine (alone or in combination with chemotherapeutic agents or vascular endothelial growth factor receptor–blocking antibody).

Results: Whereas maximum tolerated dose dacarbazine therapy did not cause significant improvement in median survival, a doublet combination of low-dose metronomic vinblastine and low-dose metronomic cyclophosphamide induced a significant increase in survival with only minimal toxicity. Furthermore, we show that the incorporation of the low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide combination with a low-dose metronomic dacarbazine regimen also results in a significant increase in survival, but not when combined with maximum tolerated dose dacarbazine therapy. We also show that a combination of metronomic vinblastine therapy and a vascular endothelial growth factor receptor 2–blocking antibody (DC101) results in significant control of metastatic disease and that the combination of low-dose metronomic vinblastine/DC101 and low-dose metronomic dacarbazine induced a significant improvement in median survival.

Conclusions: The effective control of advanced metastatic melanoma achieved by these metronomic-based chemotherapeutic approaches warrants clinical consideration of this treatment concept, given the recent results of a number of metronomic-based chemotherapy clinical trials.

Metastatic melanoma is generally associated with poor prognosis. The aggressiveness of the disease is compounded by the lack of effective treatment, with only modest progress being made over the last few decades in the discovery of chemotherapeutic drugs or other therapies, showing a meaningful clinical survival benefit in randomized clinical trials. Although single-agent chemotherapy with dacarbazine has long been the standard systemic treatment for metastatic melanoma, it is associated with low response rates of short duration (1, 2). Various combinations of chemotherapeutic agents, some of which have included dacarbazine, have also shown only very modest effects on increasing response rates and with little or no effect on improving median survival times (3–6). All of these approaches have involved conventional maximal tolerated dose regimens in which prolonged breaks are used between treatments to allow recovery from induced toxic side effects. Significant efforts have been made to enhance the efficacy of dacarbazine therapy by using it in combination with other chemotherapeutic agents. In the face of the disappointing results achieved thus far with such maximum tolerated dose approaches, it is obvious that new drugs and/or treatment strategies are needed. In this regard, the effectiveness of metronomic chemotherapy, in which drugs are given in low, minimally toxic doses at frequent regular intervals with no prolonged breaks, usually over long periods of time (7, 8), has been assessed in only a few situations in patients with metastatic or primary melanoma, thus far (9–12). Metronomic chemotherapy regimens have several potential advantages,
Translational Relevance

Despite significant efforts over the last two decades aimed at improving the efficacy of standard treatment (maximum tolerated dose of dacarbazine), there has been no significant increase in the median survival of patients suffering from metastatic melanoma. Given the lack of success achieved, a rethinking of alternative treatment strategies is needed. Using preclinical models of advanced melanoma metastasis, we show that metronomic chemotherapeutic combinations results in improved survival, which is achieved with minimal toxicity. These results compare favorably with minimal effectiveness achieved by maximum tolerated dose dacarbazine therapy (alone or in combination with other chemotherapeutic agents or a vascular endothelial growth factor receptor-blocking antibody), often accompanied by higher toxicity. Successes in preclinical setting of metastatic breast cancer have led to a clinical trial to examine the efficacy of metronomic therapy. A similar extension of the metronomic chemotherapeutic combinations presented here into the clinical setting of melanoma metastasis may be warranted.

Materials and Methods

Preclinical models of spontaneous melanoma metastasis. The 113/6-4L model of spontaneous melanoma metastasis has been previously described. The line was derived from the WM239 human melanoma cell line by serial selection of lung metastases arising in mice after orthotopic implantation of WM239 cells followed by surgical resection of the primary tumor (16). Briefly, one million 113/6-4L melanoma cells were injected subdermally into CB-17 SCID female mice, and primary tumors were allowed to develop to a size of 400 to 500 mm$^3$, at which time they were resected. Mice in the control and treatment groups were monitored regularly in accordance with the institutional guidelines stipulated by the Sunnybrook Health Sciences Centre Animal Care Committee and sacrificed when they showed signs of stress (e.g., breathing difficulty and severe weight loss). A similar approach was taken in the case of the MDA-MB-435 model of spontaneous metastasis. This cell line was initially isolated and studied as a breast cancer model but has now been recognized as representative of a human melanoma based on genomic and protein profiling (19–23). Extensive visceral metastases were observed in these models within 1 mo of removal of the primary tumor.

Impact of metronomic, maximum tolerated dose, and combination chemotherapy on survival. In the 113/6-4L model, chemotherapy was initiated 56 d after primary tumor resection, at which time extensive visceral metastatic tumor burden was present, especially evident in lungs and liver (16). Mice were treated for long periods, between 100 to 133 d in the various experiments done. The study consisted of three experiments: experiment 1 examined the efficacy of low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide versus maximum tolerated dose dacarbazine; experiment 2 examined the efficacy of maximum tolerated dose dacarbazine and low-dose metronomic dacarbazine or low-dose metronomic vinblastine or low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide could improve the efficacy of these dacarbazine-based regimens; whereas experiment 3 examined whether the incorporation of the low-dose metronomic vinblastine + DC101 combination could enhance the efficacy of maximum tolerated dose or low-dose metronomic dacarbazine regimens. Descriptions of all treatment regimens (including doses, routes of delivery, and number of mice) are outlined in Table 1.

In the case of the MDA-MB-435 metastasis model, treatment was initiated 8 d post–primary tumor removal and continued for 84 d. Mice were assigned to specific treatment groups: group 1, control (normal saline i.p. thrice per week; n = 11); group 2, low-dose metronomic vinblastine (0.33 mg/kg; i.p. thrice weekly) plus DC101 (800 mg/kg; i.p. twice weekly; n = 10); and group 3, DC101 (800 mg/kg; i.p. twice weekly; n = 10).

The efficacy of these regimens on primary tumor growth was also examined. In this case, 1 × 10$^6$ cells were implanted subdermally and primary tumors allowed to develop. Once tumors reached an average size of 200 mm$^3$, treatment was initiated and given in the regimen described including reduced acute toxic side effects, convenience when using oral drugs, and activity in some cases against refractory drug-resistant tumors; in addition, they are easily combined for prolonged periods with targeted biological therapies such as antiangiogenic drugs, with such combinations sometimes causing surprisingly robust and prolonged tumor responses (7,8,13–15).

Evaluation of metronomic chemotherapy for the treatment of advanced metastatic disease was initially done in a model using breast cancer xenografts (13). The underlying rationale was that such models might have superior predictive value for showing a clinical benefit in patients having the respective cancer with advanced metastatic disease. Our breast cancer results showed that a highly effective treatment could be achieved with the doublet combination of concurrent low-dose metronomic oral cyclophosphamide and uracil-tegafur, a fluorouracil oral prodrug (13). More recent work from our laboratory (13) indicated that such models might have superior predictive value for showing a clinical benefit in patients having the respective cancer with advanced metastatic disease. Our breast cancer results showed that a highly effective treatment could be achieved with the doublet combination of concurrent low-dose metronomic oral cyclophosphamide and uracil-tegafur, a fluorouracil oral prodrug (13). More recent work from our laboratory (13) indicated that such models might have superior predictive value for showing a clinical benefit in patients having the respective cancer with advanced metastatic disease. Our breast cancer results showed that a highly effective treatment could be achieved with the doublet combination of concurrent low-dose metronomic oral cyclophosphamide and uracil-tegafur, a fluorouracil oral prodrug (13). More recent work from our laboratory (13) indicated that such models might have superior predictive value for showing a clinical benefit in patients having the respective cancer with advanced metastatic disease.
above for control, low-dose metronomic vinblastine + DC101, and DC101. Additional treatment regimens included low-dose metronomic cyclophosphamide (20 mg/kg p.o.) + normal saline, low-dose metronomic vinblastine (0.33 mg/kg; i.p. thrice weekly) + normal saline, and low-dose metronomic cyclophosphamide (20 mg/kg p.o.) + DC101 (800 mg/kg; i.p. twice weekly). For this experiment, five mice per group were used. Mice were monitored regularly, and the experiment was terminated when tumors reached an average size of 1,500 mm³.

Statistical significance difference in survival outcomes between control and each treatment group was done using log rank test (P < 0.05 indicated statistical significance). Hazard ratio was equally done by comparing control versus each treatment group. Statistical analysis was done with the aid of GraphPad Prism4 software.

Results

Maximum tolerated dose dacarbazine chemotherapy does not improve survival of mice with advanced metastatic melanoma. Using the 113/6-4L model, we examined the efficacy of standard maximum tolerated dose dacarbazine therapy and compared the results to those achieved by observation alone. Our results show that maximum tolerated dose dacarbazine therapy is associated with minimal increases in median survival, which are not significantly different from control and associated with a high hazard ratio (P = 0.42; hazard ratio, 0.71; Fig. 1A; Table 2). This result is similar to those obtained in meta-analysis of patient outcomes comparing median survival of untreated and dacarbazine mono-

therapy patients (24) and highlights both the poor efficacy of this standard therapy, as well as the importance of developing alternative and more effective therapies.

Superior survival achieved by vinblastine/cyclophosphamide combination metronomic therapy. Preliminary examination of effectiveness of metronomic combination of low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide suggested that this therapy can also induce an increase in median survival times in our preclinical model of advanced melanoma metastasis (16). Further assessment of the efficacy of this combination showed that it mediates a significant increase in median survival when compared with control (saline alone) with an improved hazard ratio (P = 0.026; hazard ratio, 0.29; Fig. 1A; Table 2). Importantly, long-term treatment with the low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide combination was associated with minimal toxicity in metastasis-bearing mice, as denoted by a stable body weight in the treatment group (Fig. 1B). Mice in maximum tolerated dose dacarbazine groups maintained stable body weight but showed signs of distress (scruffiness and lethargy), which became more evident after each additional cycle of treatment. No such signs of distress were noted in the low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide treatment group. Survival and hazard ratio mediated by this metronomic chemotherapy regimen were superior to values achieved by maximum tolerated dose dacarbazine therapy alone.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>No. of mice</th>
<th>Treatment</th>
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<tr>
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<td>Normal saline daily in drinking water and i.p. thrice weekly</td>
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<tr>
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<td>14</td>
<td>Vbl 0.33 mg/kg i.p. thrice weekly; 20 mg/kg CTX p.o.</td>
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<tr>
<td>MTD DTIC</td>
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<td>DTIC 50 mg/kg i.p. in days 1-5 of a 21-d cycle</td>
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<th>Experiment 2</th>
<th>No. of mice</th>
<th>Treatment</th>
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<td>Saline</td>
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<td>Normal saline daily in drinking water and i.p. thrice weekly</td>
</tr>
<tr>
<td>MTD DTIC</td>
<td>9</td>
<td>DTIC 50 mg/kg i.p. in days 1-5 of a 21-d cycle</td>
</tr>
<tr>
<td>MTD DTIC + LDM Vbl</td>
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<td>DTIC 50 mg/kg i.p. in days 1-5 of a 21-d cycle; Vbl 0.33 mg/kg i.p. thrice weekly</td>
</tr>
<tr>
<td>MTD DTIC + LDM Vbl + LDM CTX</td>
<td>6</td>
<td>DTIC 50 mg/kg i.p. in days 1-5 of a 21-d cycle; Vbl 0.33 mg/kg i.p. thrice weekly; 20 mg/kg CTX p.o.</td>
</tr>
<tr>
<td>LDM DTIC</td>
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<td>DTIC 5mg/kg i.p. thrice weekly</td>
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<td>LDM DTIC + LDM Vbl</td>
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<tr>
<td>LDM DTIC + LDM Vbl + LDM CTX</td>
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<td>DTIC 5 mg/kg i.p. thrice weekly; Vbl 0.33 mg/kg i.p. thrice weekly; 20 mg/kg CTX p.o.</td>
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<th>Experiment 3</th>
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<th>Treatment</th>
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<td>Normal saline daily in drinking water and i.p. thrice weekly</td>
</tr>
<tr>
<td>LDM Vbl + DC101</td>
<td>7</td>
<td>Vbl 0.33 mg/kg i.p. thrice weekly; DC101 800 mg/kg i.p. twice weekly</td>
</tr>
<tr>
<td>LDM Vbl + LDM CTX + DC101</td>
<td>8</td>
<td>Vbl 0.33 mg/kg i.p. thrice weekly; 20 mg/kg CTX p.o.</td>
</tr>
<tr>
<td>MTD DTIC + LDM Vbl + DC101</td>
<td>8</td>
<td>DTIC 50 mg/kg i.p. in days 1-5 of a 21-d cycle; Vbl 0.33 mg/kg i.p. thrice weekly; DC101 800 mg/kg i.p. twice weekly</td>
</tr>
<tr>
<td>LDM DTIC + LDM Vbl + DC101</td>
<td>7</td>
<td>DTIC 5mg/kg i.p. thrice weekly; Vbl 0.33 mg/kg i.p. thrice weekly; DC101 800 mg/kg i.p. twice weekly</td>
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NOTE: Vinblastine/cyclophosphamide and vinblastine/DC101 combinations were examined alone or in combination with maximum tolerated dose dacarbazine and low-dose metronomic DTIC.

Abbreviations: DTIC, dacarbazine; MTD, maximum tolerated dose; LDM, low-dose metronomic; Vbl, vinblastine; CTX, cyclophosphamide.
Given the efficacy of low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide combination therapy, we next examined whether addition of this combination to standard maximum tolerated dose dacarbazine or low-dose metronomic dacarbazine therapy could improve survival. In the case of maximum tolerated dose treatment, the addition of low-dose metronomic vinblastine alone or low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide combination resulted in an increase in median survival from 147 days for maximum tolerated dose dacarbazine to 178 and 168 days, respectively. However, this increase did not reach statistical significance when compared with control ($P = 0.063; \text{Fig. 2A; Table 2}$). Mice in these groups, especially the maximum tolerated dose dacarbazine/low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide group, showed signs of toxicity associated with weight loss (Fig. 2B). This toxicity highlights one of the limitations associated with maximum tolerated dose regimens, especially when it is used in polychemotherapeutic combinations. On the other hand, the addition of low-dose metronomic vinblastine alone or low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide to low-dose metronomic dacarbazine resulted in significant increase in survival relative to control (Fig. 2C; Table 2) and moreover was associated with minimal toxicity (Fig. 2D). Interestingly, low-dose metronomic dacarbazine therapy was just as effective as the standard maximum tolerated dose dacarbazine regimen; however, the former had the added advantage of lower toxicity (Fig. 2E).

The combination of low-dose metronomic vinblastine/DC101 results in improved survival in the MDA-MB-435 model of spontaneous metastasis. We had previously shown in the MDA-MB-435 melanoma model of advanced metastatic disease that DC101 alone exerts minimal effect on survival, whereas metronomic vinblastine alone could significantly prolong

### Table 2. Summary of median survival times

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Median survival (d)</th>
<th>$P$ (vs saline)</th>
<th>Hazard ratio</th>
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<td></td>
<td></td>
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<tr>
<td>MTD DTIC</td>
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<td>0.420</td>
<td>0.71</td>
</tr>
<tr>
<td>LDM Vbl + LDM CTX</td>
<td>Undefined</td>
<td>0.026</td>
<td>0.29</td>
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<th>Experiment 2</th>
<th>Median survival (d)</th>
<th>$P$ (vs saline)</th>
<th>Hazard ratio</th>
</tr>
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<tbody>
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<td></td>
<td></td>
</tr>
<tr>
<td>MTD DTIC</td>
<td>147</td>
<td>0.120</td>
<td>0.47</td>
</tr>
<tr>
<td>MTD DTIC + LDM Vbl</td>
<td>178</td>
<td>0.063</td>
<td>0.37</td>
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<tr>
<td>MTD DTIC + DLM Vbl + LDM CTX</td>
<td>168</td>
<td>0.063</td>
<td>0.35</td>
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<tr>
<td>LDM DTIC</td>
<td>154</td>
<td>0.115</td>
<td>0.47</td>
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<tr>
<td>LDM DTIC + LDM Vbl</td>
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<td>0.35</td>
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<tr>
<td>LDM DTIC + LDM Vbl + LDM CTX</td>
<td>195.5</td>
<td>0.024</td>
<td>0.33</td>
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<th>Experiment 3</th>
<th>Median survival (d)</th>
<th>$P$ (vs control)</th>
<th>Hazard ratio</th>
</tr>
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<tr>
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<td>0.66</td>
<td>0.76</td>
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<tr>
<td>MTD DTIC</td>
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<td>0.33</td>
<td>0.59</td>
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<td>MTD DTIC + LDM Vbl + DC101</td>
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<td>0.01</td>
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<td>LDM Vbl + DC101</td>
<td>Undefined</td>
<td>0.30</td>
<td>0.49</td>
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</table>

NOTE: Metronomic combinations showed improved survival compared with control. None of the therapies that incorporated maximum tolerated dose dacarbazine resulted in significant increase in survival.
survival (25). Here, our results show that the combination of low-dose metronomic vinblastine and DC101 resulted in a dramatic suppression of metastatic disease and resulted in a significant increase in survival ($P < 0.0001$; hazard ratio, 0.04; Fig. 3A). In addition, this combination was found to be associated with significant inhibition of primary tumor growth (Fig. 3B). No such inhibition was noted by treatment with DC101 alone or in the case of low-dose metronomic vinblastine + normal saline, low-dose metronomic cyclophosphamide + normal saline, or low-dose metronomic cyclophosphamide + DC101. The last two regimens had also shown no significant effect on survival when used in the treatment of advanced metastatic disease (25).

The incorporation of metronomic vinblastine + DC101 to low-dose metronomic dacarbazine results in effective control of metastatic melanoma. We examined whether incorporation of the doublet low-dose metronomic vinblastine + DC101 combination could enhance the efficacy of maximum tolerated dose or low-dose metronomic dacarbazine chemotherapy. At the same time, we examined whether combination of low-dose metronomic vinblastine + DC101 with low-dose metronomic cyclophosphamide could result in increased antitumor effectiveness and survival. Using the 113/6-4L model, we found that low-dose metronomic vinblastine + DC101 did result in an improved outcome compared with that in control mice ($P = 0.08$; hazard ratio, 0.2; Fig. 4A; Table 2). The beneficial effect of the low-dose metronomic vinblastine + DC101 therapy did not reach statistical significance because a number of mice succumbed to toxicity mediated by long-term DC101 treatment (characterized by loss of weight; Fig. 4B). In these mice, no metastases were observed in

Fig. 2. Vinblastine and vinblastine/cyclophosphamide enhance survival when combined with low-dose metronomic dacarbazine therapy in the 113/6-4L preclinical model of advanced metastatic melanoma. A, survival curves associated with maximum tolerated dose dacarbazine as well as those in which this standard treatment was combined with vinblastine and vinblastine/cyclophosphamide in the preclinical treatment of advanced metastasis. B, body weights during the course of treatment with various regimens incorporating maximum tolerated dose DTIC. C, survival curves associated with low-dose metronomic dacarbazine as well as those in which this treatment was combined with vinblastine and vinblastine/cyclophosphamide. D, body weights during the course of treatment with various chemotherapeutic combinations incorporating low-dose metronomic dacarbazine. E, survival curves associated with low-dose metronomic dacarbazine and maximum tolerated dose dacarbazine regimens. Error bars, ±SEM.
either the chest cavity or in the central nervous system. The addition of cyclophosphamide or maximum tolerated dose dacarbazine to the low-dose metronomic vinblastine + DC101 combination did not result in enhanced survival but was associated with an exacerbation in toxicity (Fig. 4A–D; Table 2). Interestingly, a high proportion of mice (3 of 4) that succumbed to metastatic disease in the maximum tolerated dose dacarbazine/low-dose metronomic vinblastine/DC101 group showed extensive signs of central nervous system melanoma metastases; such a high frequency of central nervous system metastasis was not observed in any other treatment group. Contrary to the lack of additional activity achieved by maximum tolerated dose-based therapies, the addition of low-dose metronomic vinblastine + DC101 to low-dose metronomic dacarbazine resulted in a dramatic suppression of metastatic disease and improved median survival ($P = 0.01$; hazard ratio, 0.11; Fig. 4E; Table 2). This triplet combination also showed minimal toxicity in the treated mice (Fig. 4F) even after 13 weeks of continuous treatment.

**Discussion**

Dacarbazine has minimal impact on survival of melanoma patients. Indeed, its effectiveness has not been compared directly with observation alone, and as such, there is no clear indication about whether it improves survival (24). Given its limited therapeutic activity, dacarbazine has been combined with a number of other chemotherapeutic drugs in an effort to improve efficacy; indeed, a large number of clinical trials remain underway evaluating such combinations. The testing of these combinations has not been generally evaluated preclinically using primary tumor models, let alone those involving advanced metastatic disease. However, taking such empirical approaches into the clinic may lead to the unnecessary and premature exposure of patients to drugs for which adequate proof of activity has not been shown and for which possible toxicities have not been fully evaluated (26). Thus, preclinical evaluation of antimetastatic activity could be an essential step in the development of more effective therapeutic approaches for malignant melanoma. However, to increase relevance, such evaluation should be conducted in models that reflect the typical presentation of metastatic disease frequently seen in patients involved in clinical trials, namely, advanced, high volume metastatic disease (25, 27). In this regard, we have observed several instances wherein the outcomes of antitumor activity using primary xenograft tumor models did not correlate with antimetastatic activity (13, 16). Such a disconnect may be an important factor in the frequent disparity observed between promising preclinical therapeutic results using such primary tumor models and the subsequent, usually less impressive results observed in clinical trials.

Because they more closely reflect the clinical presentation of disease, preclinical models of advanced metastatic disease likely represent a more useful tool to examine therapies to be used for the treatment of late-stage metastases (25, 28). In support of this argument, attention is drawn to the results of a recently reported nonrandomized phase II clinical trial in metastatic breast cancer patients using a doublet metronomic chemotherapy combination, namely, daily cyclophosphamide and capalexidine, an oral 5-FU prodruk, used in combination with bevacizumab, a monoclonal anti-VEGF antibody (29). This trial was undertaken, in part, based on our previous preclinical results showing the potent activity of long-term daily low-dose cyclophosphamide with daily low-dose UFT (another 5-FU oral prodruk), which was used to treat established advanced visceral metastatic human breast cancer using a single newly developed preclinical model of advanced spontaneous metastasis (13).

The 113/6-4L model of spontaneous melanoma metastasis used in the studies described herein recapitulates all the events and steps involved in the multistep process of metastasis seen in clinic, including brain metastases (16). Using this model, we have shown that metronomic chemotherapy combination regimens are effective in controlling one of the most intractable of metastatic diseases, late-stage melanoma metastasis and moreover do so with surprisingly little associated toxicity. Furthermore, our results show that the standard maximum tolerated dose dacarbazine therapy for treatment is not as effective, and other results suggest that, even when combined with other agents (either chemotherapeutics or antiangiogenics), dacarbazine therapy provides minimal improvement in survival.

The results we have obtained should be considered in the context of the numerous clinical trials that are underway examining polychemotherapeutic approaches based on the use of maximum tolerated dose dacarbazine. The antitumor effects achieved by the low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide or low-dose metronomic dacarbazine...
dacarbazine/low-dose metronomic vinblastine/DC101 treatments suggest that improved survival in melanoma patients might be achieved by such combination metronomic therapy approaches and, moreover, with lesser toxicity. Our data suggest that consideration might be given to combining antiangiogenic agents and dacarbazine using a metronomic regimen, perhaps immediately following upfront conventional dacarbazine chemotherapy alone or in combination with an antiangiogenic agent. The feasibility of such metronomic chemotherapy might be improved by substituting dacarbazine (an agent administered i.v.) with orally bioavailable agents such as temozolomide, an agent that is equally effective as dacarbazine in the treatment of malignant melanoma but also shows superior penetration into the brain parenchyma (2, 30).

It is interesting to note that the effectiveness achieved by the metronomic chemotherapeutic regimens that we have examined (low-dose metronomic vinblastine/low-dose metronomic cyclophosphamide in this study and low-dose metronomic cyclophosphamide/UFT in previous breast cancer studies) against advanced metastatic disease is often contrasted by their relative ineffectiveness against primary tumors (13, 16). This disparity may be ascribed to differential effects mediated by metronomic therapy against endothelial cells in specific sites (e.g., lung parenchyma metastases versus subdermal primary tumor). In turn, this divergence in sensitivity may be attributed to the heterogeneity of endothelial cells present in different organ sites, as well as to differences in the microenvironment itself (e.g., presence of distinct growth factors, cytokines, etc.). Given that the primary target of metronomic chemotherapy schedules is presumed to be the endothelial cell population of the neovasculature of the tumor, it reasons that this antiangiogenic effect can be enhanced by the incorporation of “dedicated” antiangiogenic agents. In this regard, the effective suppression of both metastatic disease and primary tumor growth that was mediated by the combination of metronomic vinblastine and the VEGF receptor–targeting antibody (DC101) suggests that this

![Fig. 4](https://www.aacrjournals.org)
Combination therapy approach is more effective against tumors growing in different sites.

In addition to the current lack of effective therapies for advanced melanoma metastasis, there are also few alternatives for adjuvant therapy. Presently, interferon-α-2b is the only Food and Drug Administration–approved agent for the treatment of melanoma patients who are free of disease but at high risk for recurrence (31). However, despite the initial promise, this agent has been shown to induce only a small improvement on overall survival; moreover, this minimal beneficial effect is achieved with high levels of toxicity (31, 32). Given the efficacy and minimal toxicity of the regimens noted herein, consideration should be given to evaluating the efficacy of the metronomic chemotherapy approach in the adjuvant setting.

Disclosure of Potential Conflicts of Interest

R.S. Kerbel holds a Tier I Canada Research Chair in Tumor Biology, Angiogenesis, and Antiangiogenic Therapy.

Acknowledgments

We thank ImClone Systems for their gift of the VEGF receptor targeting antibody, DC101.

References

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