Re: Macrophage Role in the Anti-Prostate Cancer Response to One Class of Antiangiogenic Agents

As reported in a recent article in the Journal, Joseph and Isaacs (1) tried to combine Linomide® with other antiangiogenic agents known to inhibit tumor-associated macrophages (TAMs) to evaluate their joint antitumor effects. Unfortunately, the combination of Linomide with other antiangiogenic agents did not result in potentiation of therapeutic efficacy. Instead, one of these agents, pentoxifylline, inhibited the an-
titumor effects of Linomide. In an editorial accompanying the article by Joseph and Isaacs, Wahl and Kleiman (2) suggested, on the other hand, that tumor therapy protocols that combine Linomide with the antiangiogenic cytokine interleukin 12 (IL-12)—which induces interferon-gamma release and activates rather than inhibits TAMs—would be worth pursuing.

The results of our present studies demonstrate the pertinence of this suggestion. In a murine melanoma model, in which the antitumor activity of Linomide has already been demonstrated (3), we show that administration of IL-12 in combination with Linomide results in potentiated antitumor effects over single-agent therapy (two-sided test) (Fig. 1).

To confirm that IL-12 and Linomide, used alone or in combination, exert the antiangiogenic effects that were expected on the basis of previous reports, we used a tumor-induced angiogenesis model. This model was proven effective in a previous study of IL-12 and tumor necrosis factor (5). Short-term (3 consecutive days) intraperitoneal administration of either 0.1 μg IL-12 or 400 mg Linomide resulted in significant inhibition of blood vessel formation at the site of B16F10 murine melanoma cell injection (16.4 blood vessels per microscope field [95% confidence interval {CI} = 14.7–18.1 vessels] and 16.0 blood vessels per field [95% CI = 15.2–16.8 vessels] in IL-12 and Linomide-treated mice, respectively) as compared with diluent-injected controls (18.7 vessels per field [95% CI = 17.8–19.5 blood vessels]; two-sided \( P = 0.007 \) for IL-12 versus control group; two-sided \( P = 0.0003 \) for Linomide versus control group; Mann–Whitney \( U \) test). Combined treatment with IL-12 and Linomide was statistically significantly more effective in inhibiting the formation of new blood vessels than was the treatment with either agent alone (13.7 blood vessels per field [95% CI = 12.3–15.1 vessels]; two-sided \( P = 0.012 \) for IL-12 versus combined treatment group; two-sided \( P = 0.0028 \) for Linomide versus combined treatment group).

Our results demonstrate potentiation of antitumor and antiangiogenic effects for combination therapy with IL-12 and Linomide in a murine melanoma model. These observations would appear to be clinically interesting because antitumor efficacy of both these agents is currently being evaluated in clinical trials (6,7).

**Fig. 1.** Effects of treatment with interleukin 12 (IL-12) and/or Linomide® on B16F10 melanoma growth (IL-12 was a gift from the Genetics Institute Inc., Boston, MA, and Linomide was a gift from Pharmacia and Upjohn, Lund, Sweden). B6D2F1 mice were inoculated with 1 × 10⁶ melanoma cells in the footpad of the right hind limb and treated intratumorally with 0.1 μg IL-12 for 7 consecutive days and/or intraperitoneally with 400 mg/kg of Linomide for 21 days starting on day 7 after inoculation of tumor cells. As a control, the following diluents were used: 0.1% bovine serum albumin–phosphate-buffered saline (PBS) administered intratumorally for 7 consecutive days and 0.01% Tween in PBS administered intraperitoneally for 21 consecutive days. Groups consisted of seven to eight mice. Doses of IL-12 and Linomide were established in previous experiments (data not shown). Tumor growth was monitored as described previously (4). Tumor volumes are expressed as means with 95% confidence intervals. * = two-sided \( P < 0.05 \) as compared with all other groups (Mann–Whitney \( U \) test). \( \star \) = control; \( \bullet \) = IL-12; \( \square \) = Linomide; and \( \triangledown \) = IL-12 + Linomide.

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**RESPONSE**

We thank Dabrowska and colleagues for their efforts in demonstrating that the combination of interleukin 12 (IL-12) with Linomide® potentiated the antiangiogenic and antitumor effects of each monotherapy in a murine melanoma model. IL-12 released by activated macrophages is an inducer of interferon gamma (IFN-γ) and indirectly of inter-
feron IFN-γ-inducible protein (IP-10), which inhibits angiogenesis. IP-10 and Linomide inhibit different steps in the angiogenic process. IP-10 inhibits the differentiation of endothelial cells into tube-like structures but does not affect endothelial cell proliferation, migration, or invasion (1). On the other hand, Linomide inhibits endothelial cell proliferation, migration, and invasion (2). In addition, IL-12 inhibits tumor cell production of vascular endothelial growth factor (VEGF) through IFN-γ while Linomide does not inhibit VEGF production by tumor cells (3,4). Thus, Linomide and IL-12 appear to have different, but complementary, mechanisms of action, which results in potentiated antitumor effects when they are combined.

It is interesting to note that the authors administered Linomide intraperitoneally at a dose of 400 mg/kg. We have demonstrated better antiangiogenic and antitumor effects in our human prostate cancer xenograft models when Linomide was administered orally at a much lower dose (5). This observation indicates that the oral route of administration is more effective than the parental route for mice. The authors could have achieved optimal antitumor effects had they administered Linomide in drinking water.

It is also interesting to note that, when Linomide or IL-10 monotherapy was terminated on day 21 after tumor inoculation, the subsequent tumor growth was enhanced. In contrast, in the combination group, the subsequent rate of tumor growth was not enhanced following termination of treatments. We also have observed similar effects with Linomide in our rodent prostate cancer models, suggesting that Linomide needs to be administered at least intermittently (6).

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EDITOR’S NOTE

Larry M. Wahl and Hynda K. Kleinman declined to respond to Anna Dabrowska et al.’s correspondence.