

Anti-IL-23 Monoclonal Antibody Synergizes in Combination with Targeted Therapies or IL-2 to Suppress Tumor Growth and Metastases

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Abstract

Immunosuppressive barricades erected by tumors during the evolution of immune escape represent a major obstacle to many potentially effective cancer therapies and vaccines. We have shown that host interleukin (IL)-23 suppresses the innate immune response during carcinogenesis and metastasis, independently of effects on the proinflammatory cytokine IL-17A. Based on these findings, we envisioned that IL-23 neutralization might offer a promising strategy to modulate immunosuppression, particularly in combination with immunostimulatory agents. Here we show that by itself a neutralizing monoclonal antibody (mAb) to IL-23 suppressed early experimental lung metastases in the B16F10 mouse model of melanoma and also modestly inhibited the subcutaneous growth of primary tumors. These antitumor effects were respectively mediated by natural killer cells or CD8⁺ T cells. More notably, combinatorial treatments of anti-IL-23 mAb with IL-2 or anti-erbB2 mAb significantly inhibited subcutaneous growth of established mammary carcinomas and suppressed established experimental and spontaneous lung metastases. Overall, our results suggest the potential of anti-human IL-23 mAbs to improve the immunostimulatory effects of IL-2 and trastuzumab in the current management of some advanced human cancers. *Cancer Res*; 71(6); 2077–86. ©2011 AACR.

Introduction

The role of chronic inflammation has been implicated in the initiation and progression of a range of malignancies including lung, liver, and gastrointestinal carcinomas (1, 2). Proinflammatory and immunosuppressive factors that can aid in this process include interleukin (IL)-1 β , IL-6, TGF- β , and the bioactive lipid prostaglandin E2 (1). Indeed, tumor immunosuppression remains one of the major obstacles to many potentially effective cancer therapies and vaccines (3, 4). Studies from mouse models of *de novo* tumorigenesis and clinical data from cancer patients have shown tumor-specific T cell generation and infiltration into tumor sites (4, 5). Furthermore, strategies such as active immunization results in some patients having up to 30% of all their circulating CD8⁺ T cells capable of reactivity to tumors (6). Nevertheless, the presence of tumor-specific T cells alone is generally not

enough to result in tumor regression, as tumors induce a tolerant microenvironment and have a range of immunosuppressive mechanisms that can suppress antitumor immunity (7). Thus, modulation of tumor-induced immunosuppression will be important in allowing the optimal antitumor activity of immunotherapies to be achieved.

Recently, the heterodimeric cytokine IL-23, formed by linkage of the p40 to a p19 subunit, has emerged as a new player in promoting tumor growth and development (8). Langowski and colleagues described that IL-23p19-deficient (IL-23p19^{-/-}) mice were very resistant to 7,12-dimethylbenz[*a*]anthracene (DMBA)/12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced skin papillomas (8) and suggested that CD8⁺ T cells were one of the targets of IL-23 suppression since a significantly greater CD8⁺ infiltrate was observed in the carcinogen-treated skin of IL-23p19-deficient mice than in wild-type mice. More recently, we published a report confirming Langowski's claim that IL-23p19^{-/-} mice were very resistant to DMBA/TPA-induced skin papillomas and further demonstrated that these mice were also highly resistant to 3'-methylcholanthrene-induced fibrosarcomas and 3 different models of experimental tumor metastases (9). Importantly, in these tumor models where host natural killer (NK) cells were the main effectors in controlling disease, we reported for the first time that IL-23 suppressed natural and cytokine-activated NK cell cytotoxicity and cytokine functions. Although IL-23 has generally been associated with the proinflammatory cytokine IL-17A, which is the signature cytokine produced by Th17 cells (reviewed in ref. 10), interestingly, we did not see an important role for host IL-17A in

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many of the tumor models where IL-23 had tumor-promoting activity (9, 11).

Neutralization of host IL-23 may offer a promising new approach for modulating tumor-induced immunosuppression, particularly when used in combination with immunostimulatory agents. In this study, we have shown that a neutralizing mouse monoclonal antibody (mAb) to mouse IL-23 (α -IL-23) alone suppressed early experimental and spontaneous tumor metastases and, when used in combination with IL-2 or anti-erbB2 mAb treatment, significantly inhibited established experimental and spontaneous lung metastases or established primary breast cancer, respectively.

Materials and Methods

Mice

Inbred C57BL/6 wild-type (WT), C57BL/6 IL-12p35-deficient (IL-12p35^{-/-}), C57BL/6 IL-12p40-deficient (IL-12p40^{-/-}), C57BL/6 IL-23p19-deficient (IL-23p19^{-/-}), C57BL/6 IL-17A-deficient (IL-17A^{-/-}), C57BL/6 perforin-deficient (pfp^{-/-}), C57BL/6 IFN- γ -deficient (IFN- γ ^{-/-}), and BALB/c WT mice were bred and maintained at the Peter MacCallum Cancer Centre (Peter Mac) as described previously (9). Mice 6 to 14 weeks old were used in all experiments. BALB/c or C57BL/6 female mice were used for all experiments with the 4T1.2 or E0771 tumors, respectively, and all experiments were carried out in accordance with guidelines set out by the Peter Mac Animal Experimental Ethics Committee guidelines.

Tumor models

Experimental tumor metastases model. C57BL/6 WT or gene-targeted mice were inoculated i.v. with the indicated doses of B16F10 melanomas cells (from ATCC). Mice were monitored and culled 14 days after tumor inoculation and lung metastases were quantified as described previously (12).

Subcutaneous tumor model: Groups of C57BL/6 WT, IL-23p19^{-/-} or IL-12p40^{-/-} mice were injected subcutaneously with 1×10^6 EG7 thymoma cells (originally provided by Frank Carbone, University of Melbourne).

Spontaneous tumor metastasis model: The indicated doses of E0771 or 4T1.2 mammary carcinoma cells [originally provided by Robin Anderson (Peter Mac)] were injected into the mammary fat pad of C57BL/6 WT or BALB/c WT mice.

Reagents

Anti-AGP3 [control Ig (cIg), 4D2], anti-IL-23 (16E5), and anti-IL-12p40 (C17.8) were all grafted on the same murine IgG1 background and kindly provided by AMGEN Incorporated. Some groups of mice were treated with control Ig (MAC-4), anti-CD8 α (53.6.7; produced in-house), anti-CD8 β (produced in-house), anti-CD4 (GK1.5; produced in-house), or anti-asialoGMI (Wako Chemicals), as indicated in the legends to deplete cell subsets as previously described (9). To examine cytokine immunotherapy of metastases, some groups of mice received either PBS or recombinant IL-2 (Chiron Corporation) at the indicated time points and dose in combination with

anti-AGP3, anti-IL-23, anti-IL-12p40, or anti-CD137 (3H3; produced in-house).

Statistical analysis

Significant differences in metastases or survival were determined by the Mann-Whitney *U* test and the log-rank sum test. Values of *P* < 0.05 were considered significant.

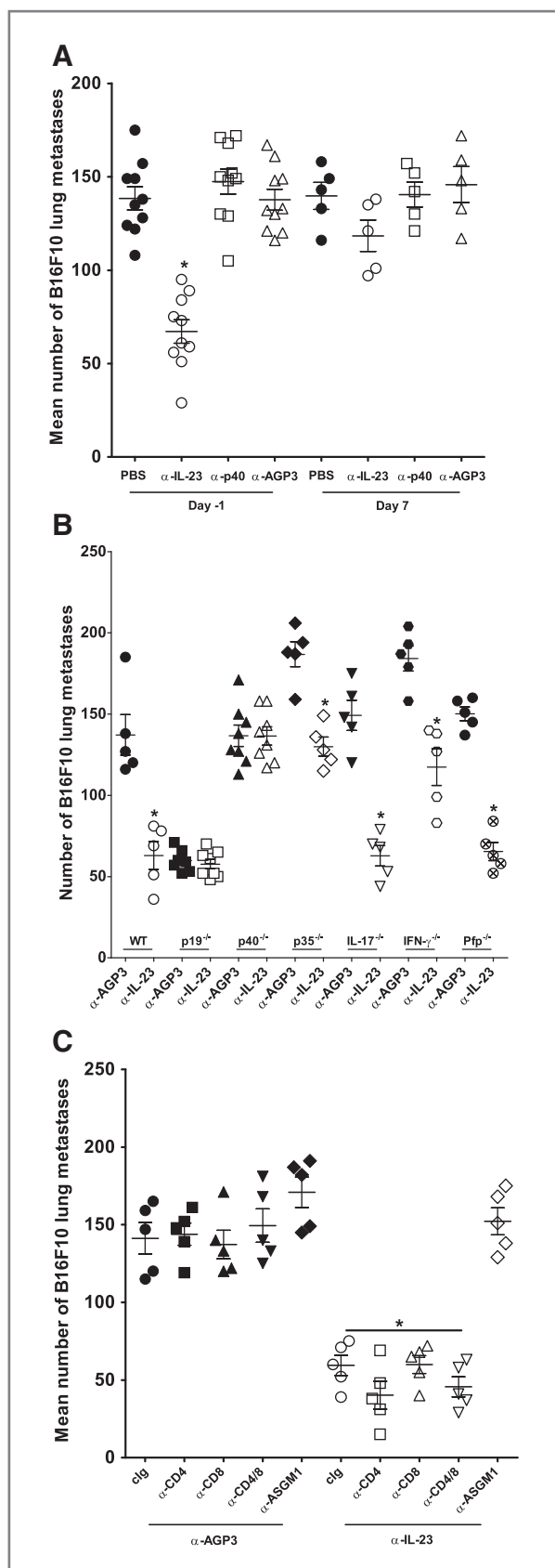
Results

Neutralization of IL-23 enhances NK-cell-mediated control of established experimental B16F10 lung metastases

We have previously shown that IL-23p19^{-/-} mice have significantly less experimental B16F10 lung metastases than in WT mice (9). Therefore, we first assessed the effects of a neutralizing mouse anti-mouse IL-23 (α -IL-23) mAb in this model (Fig. 1). A single treatment of WT mice with α -IL-23 mAb given 1 day before tumor inoculation resulted in significantly fewer lung metastases than in mice treated with control Ig (cIg (α -AGP3, on the same mouse backbone; *P* = 0.0002), with the level of tumor suppression similar to that we have previously observed in IL-23p19^{-/-} mice (ref. 9; Fig. 1A and B). Furthermore, a single dose of α -IL-23 mAb treatment also reduced day 7 established B16F10 lung metastases, although not significantly (*P* = 0.22). In contrast, α -p40 mAb treatment, which neutralizes both IL-23 and IL-12, did not suppress lung metastases whether given at day 1 or 7, showing that loss of IL-12 countered the loss of IL-23. This result was consistent with the known comparative metastases in IL-12p35^{-/-}, IL-12p40^{-/-}, and IL-23p19^{-/-} mice (ref. 9; Fig. 1B). Notably, anti-IL-23 was effective in WT, pfp^{-/-}, and IL-17A^{-/-} mice but not surprisingly totally ineffective in IL-23p19^{-/-} or IL-12p40^{-/-} mice. Anti-IL-23 mAb was partially effective in IFN- γ ^{-/-} and IL-12p35^{-/-} mice, suggesting that IL-23 was acting in opposition to IFN- γ and IL-12 and was in large part responsible for the higher metastatic load observed in these mice (Fig. 1B). NK cells were the major cell population responsible for the antitumor activity of α -IL-23 mAb treatment in the B16F10 experimental lung metastases model, as suppression was lost following depletion of NK cells with anti-ASGMI (Fig. 1C). Similar levels of metastases suppression were observed in α -IL-23 mAb-treated mice that were injected with anti-CD4 and/or anti-CD8 mAb compared with cIg-treated groups (*P* = 0.0079), showing that α -IL-23 mAb control of B16F10 lung metastases was independent of CD4⁺ and CD8⁺ T cells.

IL-23 suppresses CD8⁺ T-cell control of subcutaneous EG7 tumor growth

We next wanted to determine whether α -IL-23 mAb could also promote adaptive immunity. We therefore tested the therapeutic efficacy of α -IL-23 mAbs against the subcutaneous growth of EG7 (EL4-OVA), which is known to be naturally controlled by CD8⁺ T cells (ref. 13; Fig. 2). The growth of EG7 was significantly inhibited in mice that were treated early with α -IL-23 mAb (days -1, 3, 10, 14, 17, and 21) compared with similar groups of mice that were treated with



control α -AGP3 mAb ($P = 0.0002$; Fig. 2A). Inhibition by α -IL-23 mAb was equivalent to that observed in IL-23p19^{-/-} mice. Similar to observations in the B16F10 metastases model, no growth inhibition of EG7 tumor was observed in WT mice injected with α -p40 mAb or in IL-12p40^{-/-} mice. Depletion of CD8⁺ T cells in α -IL-23 mAb-treated WT mice completely abrogated the therapeutic effect (Fig. 2B). This loss of tumor growth inhibition was similarly observed in IL-23p19^{-/-} mice injected with anti-CD8 mAbs. In contrast, EG7 tumor growth was still reduced in WT mice treated with α -IL-23 mAbs or IL-23p19^{-/-} mice that were depleted of NK cells. Interestingly, treatment with α -IL-23 mAb from day 6 in WT mice bearing more established EG7 tumors could not significantly attenuate tumor growth (Fig. 2C). Collectively, our data suggested that neutralization of IL-23 promotes either innate or adaptive immunity depending on the tumor model.

Anti-IL-23 mAb and IL-2 immunotherapy in combination reduces established experimental B16F10 lung metastases

We next wanted to determine whether other immunotherapies could be improved in combination with the α -IL-23 mAb. We had previously observed that early, high-dose IL-2 treatment of IL-23p19^{-/-} mice injected with a high dose of B16F10 tumors had significantly reduced lung metastases compared with similar groups of tumor-bearing IL-23p19^{-/-} mice not treated with IL-2 (9). Groups of WT mice injected i.v. with a high dose of B16F10 tumors (7.5×10^5 cells) and treated with α -IL-23 mAb and PBS had a trend toward decreased lung metastases compared with similar groups of mice treated with clg or α -p40 and PBS ($P = 0.056$; Fig. 3A). Strikingly, there was a significant decrease in lung metastases in mice that were treated with α -IL-23 and IL-2 (10^5 U; $P = 0.0079$) compared with similar groups of IL-2-treated mice that were injected with α -AGP3 or α -p40 (Fig. 3A). The increased therapeutic effect of combining α -IL-23 mAb and IL-2 immunotherapy was most obvious when the dose of IL-2 administered was decreased from 10^5 to 10^4 units (Fig. 3B).

Given that α -IL-23 mAb treatment alone resulted in modest suppression of established B16F10 lung metastases, we next wanted to assess whether the combination of α -IL-23 mAb and IL-2 immunotherapy could result in improved therapeutic efficacy. In addition, we also wanted to determine the optimal

Figure 1. NK-cell-mediated control of B16F10 experimental lung metastases after anti-IL-23 therapy. A, groups of C57BL/6 WT mice ($n = 5-10$) were injected i.v. with 2×10^5 B16F10 melanoma cells on day 0. Mice were treated with control Ig (α -AGP3), anti-IL-12p40 (α -p40), or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) on day 1 (A and C) or day 7 (A) relative to tumor inoculation. B, groups of C57BL/6 WT or gene-targeted mice ($n = 5-8$) as indicated were injected i.v. with 2×10^5 B16F10 melanoma cells on day 0. Mice were treated with control Ig (α -AGP3) or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) on day 0. C, mice were additionally depleted of CD8⁺ T cells (α -CD8) or NK cells (α -ASGM1) by treatment with antibodies (100 μ g i.p.) on days -1, 0, and 7 relative to tumor inoculation. Fourteen days after tumor inoculation, the lungs of these mice were harvested and fixed and the colonies were counted and recorded for individual mice as shown. Asterisks indicate the groups that are significantly different from PBS treated or clg-treated mice, using the Mann-Whitney rank-sum test (*, $P < 0.05$).

time point for the administration of these two agents (Fig. 4). Similar to the experiment above, groups of WT mice were injected i.v. with a high dose of B16F10 tumors on day 0 (Fig. 4A). As expected, treatment with both α -IL-23 mAb and IL-2 on day 1 prior to tumor inoculation resulted in the best level of suppression compared with similar groups of mice

that were treated with cIg and IL-2 on day -1 ($P = 0.0079$; Fig. 4A). Importantly, combination treatment in groups of mice also significantly reduced day 7 established B16F10 lung metastases compared with similar groups of mice that received either IL-2 or α -IL-23 mAb on day 7 ($P = 0.0465$). Interestingly, no decrease in lung metastases was observed in groups of mice that were first treated with α -IL-23 mAb on day -1 followed by IL-2 therapy on day 7 or those that were treated with IL-2 on day -1 followed by α -IL-23 mAb on day 7. We next assessed whether the order of treatment affected therapeutic efficacy in WT mice bearing established day 7 B16F10 lung metastases (Fig. 4B). Generally, IL-2 treatment of tumor-bearing mice in combination with α -IL-23 mAb therapy administered at the stated time points could all significantly decrease B16F10 lung metastases compared with similar groups of mice treated with α -AGP3 ($P = 0.012$). However, neutralization of IL-23 with α -IL-23 mAb on days 5 to 7 followed by IL-2 therapy on day 7 resulted in the optimal decrease in lung metastases ($P = 0.016$) compared with those that were treated with IL-2 on day 7 and α -IL-23 mAb on either day 7 or days 7 to 9. Indeed, the schedule of α -IL-23 mAb treatment on days 5 to 7 followed by IL-2 therapy on day 7 generally resulted in the best therapeutic effect as observed in 3 other different experimental lung metastases models (Supplementary Fig. 1).

Since NK cells control B16F10 lung metastases, we next sought to determine whether NK cell proportions, numbers, and cytotoxic function were enhanced in tumor-bearing mice following α -IL-23 mAb and/or IL-2 treatment in combination (Supplementary Fig. 2). Analysis of these parameters revealed that all treatments did not dramatically modulate NK cell proportions and NK cell numbers in the lungs or spleen other than a trend toward increased NK cell proportion and number in the lungs of IL-2-treated mice and a lower proportion of NK cells in the lungs of α -IL-23 mAb-treated mice (Supplementary Fig. 2A–D). Not surprisingly, IL-2 dramatically increased the cytotoxic activity of both lung and spleen mononuclear cells (MNC; Supplementary Fig. 2E and F). However, more importantly, α -IL-23 mAb significantly increased the cytotoxic activity of lung MNCs, but not spleen MNCs, in mice additionally treated with cIg (α -AGP3) or IL-2. When correcting for NK cell proportion, this increase in cytotoxicity is even greater following α -IL-23 mAb treatment. Overall, these data suggested that

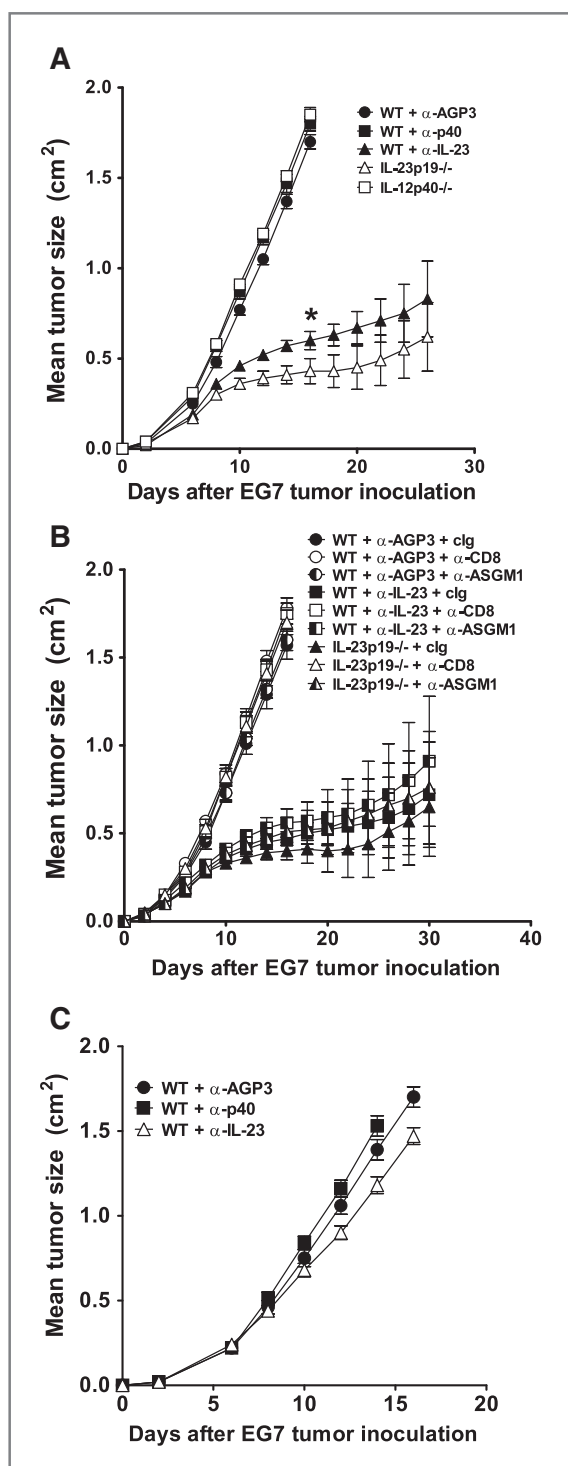


Figure 2. CD8⁺ T cells can inhibit subcutaneous EG7 tumor growth following early IL-23 neutralization. A–C, groups of C57BL/6 WT, IL-23p19^{-/-}, or IL-12p40^{-/-} mice ($n = 5$ –10/group) were injected subcutaneously with 1×10^6 EG7 thymoma cells. A and B, tumor-bearing WT mice were also treated with control isotype anti-AGP3, anti-IL-12p40, or anti-IL-23 mAbs (500 μ g i.p.) on days -1, 3, 7, 10, 14, 17, and 21. B, tumor-bearing WT mice and IL-23p19^{-/-} mice were additionally depleted of CD8⁺ T cells (α -CD8) or NK cells (α -ASGM1) by treatment with antibodies (100 μ g i.p.) on days -1, 0, 7, and 14 relative to tumor inoculation. C, tumor-bearing WT mice were treated with control isotype anti-AGP3, anti-IL-12p40, or anti-IL-23 antibodies (500 μ g i.p.) on days 6, 10, and 13. Mice were monitored for tumor growth (calculated by the product of 2 perpendicular axes), and data are presented as the mean tumor size (cm^2) \pm SEM. Statistical differences between control Ig treated mice or anti-IL-23-treated mice or tumor-bearing WT or IL-23p19^{-/-} mice were determined by the Mann-Whitney rank-sum test (*, $P < 0.05$).

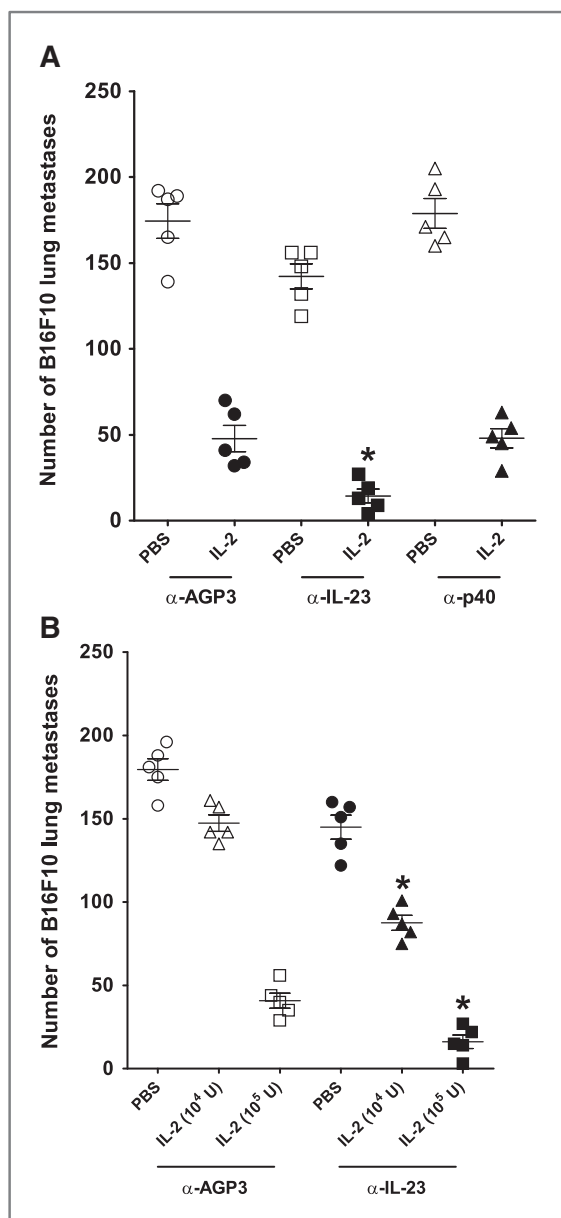


Figure 3. Anti-IL-23 enhances dose-dependent IL-2 immunotherapy of B16F10 experimental lung metastases. A and B, groups of C57BL/6 WT mice ($n = 5$) were injected i.v. with 7.5×10^5 B16F10 melanoma cells on day 0. A, mice were treated with PBS or IL-2 (10^5 U) i.p. in combination with control Ig (α -AGP3), anti-IL-12p40 (α -p40), or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) on day -1. B, mice were treated with PBS or 2 different doses of IL-2 (10^4 or 10^5 U) in combination with either control Ig (α -AGP3) or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) on day -1. Fourteen days after tumor inoculation, the lungs of these mice were harvested and fixed and the colonies were counted and recorded for individual mice as shown. Asterisks indicate the groups that are significantly different from PBS and α -AGP3-treated or IL-2 and α -AGP3-treated mice using the Mann-Whitney rank-sum test (*, $P < 0.05$).

α -IL-23 mAb and IL-2 therapy can impact on a range of established experimental lung metastases and that the regimen of first neutralizing IL-23, followed by IL-2 therapy to activate NK cells, may produce the best therapeutic outcome.

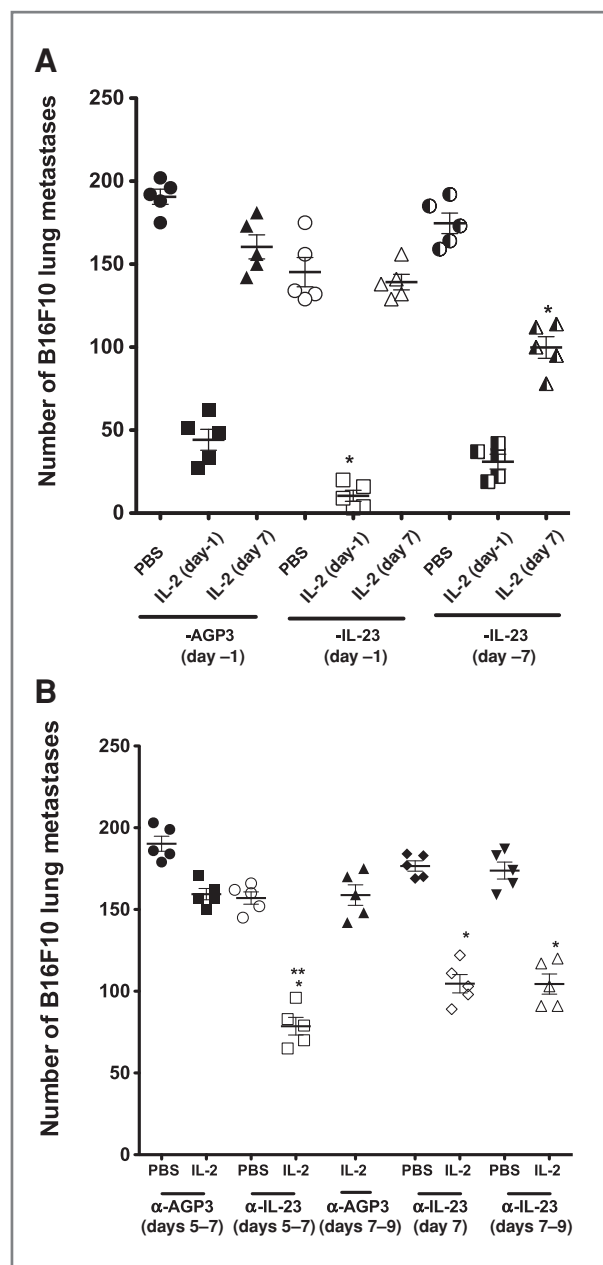


Figure 4. Anti-IL-23 and IL-2 immunotherapy suppress established B16F10 experimental lung metastases. A and B, groups of C57BL/6 WT mice ($n = 5$) were injected i.v. with 7.5×10^5 B16F10 melanoma cells on day 0. A, mice were treated i.p. with PBS (day -1) or IL-2 (10^5 U; day -1 or 7) in combination with either control Ig (α -AGP3) or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) at the time points indicated. B, mice were treated with PBS or IL-2 (10^5 U) on day 7 in combination with either control Ig (α -AGP3) or anti-IL-23 (α -IL-23) mAbs (500 μ g i.p.) at the time points indicated. Fourteen days after tumor inoculation, the lungs of these mice were harvested and fixed and the colonies were counted and recorded for individual mice as shown. Statistics were performed using the Mann-Whitney rank-sum test (*, $P < 0.05$; **, $P < 0.05$). A, asterisks indicate the α -IL-23/IL-2 groups that are significantly different from α -AGP3/IL-2-treated mice (day -1 or 7; *). B, asterisks indicate the α -IL-23/IL-2 groups that are significantly different from PBS-treated or α -AGP3-treated mice (*) or between various groups that treated with IL-2 and α -IL-23 (day 5-7 vs. day 7 or days 7-9; **).

IL-2 and anti-IL-23 prolong the survival of mice from spontaneous mammary metastases post-primary tumor resection and require both NK and CD8⁺ T cells

Having shown that α -IL-23 mAbs alone can impact on early experimental lung metastases, we next wanted to determine the therapeutic efficacy of α -IL-23 mAbs on spontaneous metastases (Fig. 5). Groups of C57BL/6 mice were injected in the mammary fat pad with 5×10^5 EO771 mammary carcinoma cells. These mice were treated i.p. twice weekly for 4 weeks with either α -AGP3 or α -IL-23 mAbs. Twenty

days after tumor inoculation, the primary tumors had grown equivalently (Fig. 5A; mean = 0.70–0.80 cm² in both groups of mice) and, in both groups, the primary tumors were resected. Mice were sacrificed at day 35, and lungs were harvested and the colonies were counted and recorded for individual mice as shown in Figure 5B. Clearly, mice that were treated with α -IL-23 mAbs had significantly lower number of lung metastases than in α -AGP3-treated group ($P = 0.0015$). Next, we wanted to assess the therapeutic efficacy of combining α -IL-23 mAb and IL-2 therapy on

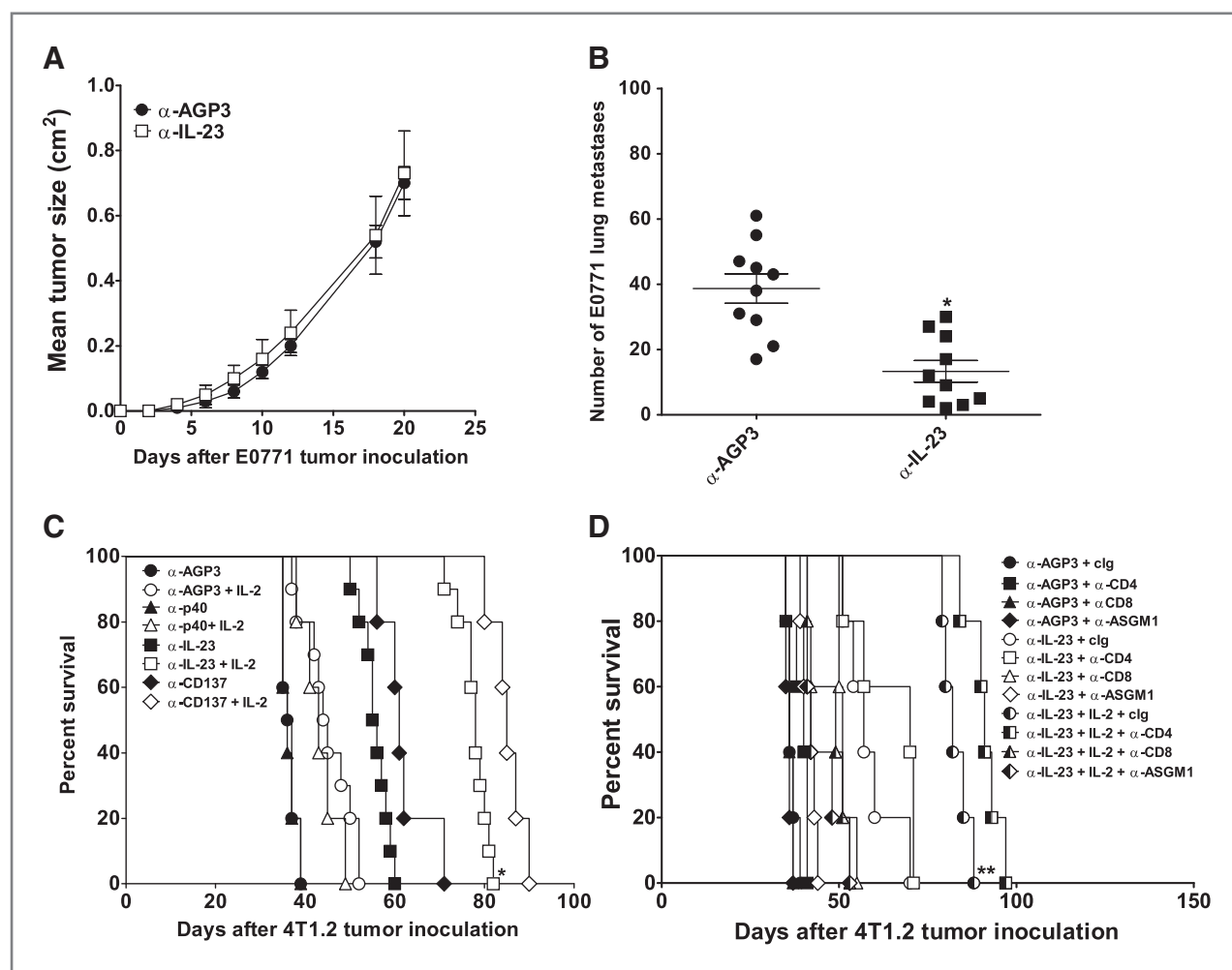


Figure 5. Anti-IL-23 immunotherapy prolongs the survival of mice from spontaneous metastases post primary tumor resection. Groups of 10 C57BL/6 mice were injected in the mammary fat pad with 5×10^5 EO771 mammary carcinoma cells. Groups of mice were treated i.p. twice weekly for 4 weeks with either control Ig (α -AGP3) or anti-IL-23 (α -IL-23) mAbs (500 μ g). A, primary tumor growth was measured for 20 days, and 20 days after tumor inoculation, the primary tumors (mean = 0.70–0.80 cm² in both groups of mice) were resected. B, mice were sacrificed at day 35, lungs were harvested, and the colonies were counted and recorded for individual mice as shown. Asterisks indicate that the α -IL-23 group was significantly different from control anti-AGP3-treated mice, using the Mann-Whitney rank-sum test (*, $P < 0.05$). C and D, groups of 5–20 BALB/c WT mice were injected in the mammary fat pad with 2×10^4 4T1.2 mammary carcinoma cells, and 28 days later, the primary tumors (mean = 0.70–0.83 cm² in all groups of mice) were resected and groups of mice were then treated on days 29, 30, and 31 i.p. with either control Ig (α -AGP3), anti-IL-12p40 (α -p40), or anti-IL-23 (α -IL-23) mAbs (500 μ g) or anti-CD137 (100 μ g) alone or in combination with IL-2 (10⁵ U i.p.; day 31). C, mice were additionally depleted of CD4⁺ T cells (α -CD4), CD8⁺ T cells (α -CD8), or NK cells (α -ASGM1) by treatment with antibodies (100 μ g i.p.) on days 28, 29, 36, 43, 50, 57, 61, and 71 following tumor inoculation. Statistical differences in survival between tumor-bearing mice treated with α -IL-23 or α -IL-23 and IL-2 were determined by the log-rank sum test (*, $P < 0.05$). Statistical differences in survival between tumor-bearing mice treated with α -IL-23/IL-2 and cIg were compared with α -IL-23/IL-2 and α -CD8 or α -ASGM1 (determined by the log-rank sum test; **, $P < 0.05$).

established spontaneous lung metastases (Fig. 5C and D). Groups of BALB/c WT mice were inoculated in the mammary fat pad with the 4T1.2 mammary carcinoma cell line and 28 days later, the primary tumors were resected. Treatment of these mice with α -IL-23 mAbs and IL-2 commenced after resection and resulted in significant survival over similar groups of mice that were treated with clg or α -p40 and IL-2 or α -IL-23 alone ($P < 0.0001$; Fig. 5C). This increase in survival was comparable with mice that were treated with α -CD137 mAb and IL-2. In some experiments, groups of mice with resected tumors treated with α -IL-23 mAbs and IL-2 were additionally depleted of CD4⁺, CD8⁺, or NK cells to determine which immune subsets were involved in enhancing the survival of these mice (Fig. 5D). Clearly, both CD8⁺ and NK cells were required, as depletion of either subset resulted in a loss of survival after treatment with α -IL-23 mAb and IL-2. In contrast, CD4⁺ T-cell depletion did not affect the efficacy of the combination treatment but, on the contrary, seemed to further improve survival ($P = 0.0127$). Treatment of groups of mice with α -IL-23 mAbs alone also resulted in increased survival compared with those treated with α -AGP3, but this was clearly not as effective as that achieved by the combination therapy.

IL-23 neutralization in combination with anti-erbB2 treatment

While we have shown that the combination of IL-2 and α -IL-23 mAb synergizes in a range of tumor metastases models, the proof of principle that neutralization of IL-23 followed by activation of innate immune cells suggests that other more commonly used agents that engage NK cells may also be suitable for use in combination with α -IL-23 mAbs. It has previously been shown that α -erbB2 mAb alone can impact on tumor growth and this process was dependent on antibody-dependent cell cytotoxicity and NK cells (14, 15). We therefore assessed the therapeutic efficacy of α -erbB2 (7.16.4; prepared in-house as described in ref. 15) and α -IL-23 mAbs in BALB/c mice bearing day 15 established subcutaneous H2N100 mammary tumors that express oncogenic rat erbB2 (Fig. 6). While, treatment of tumor-bearing mice with α -IL-23 mAbs or α -erbB2 resulted in either modest or good suppression of tumor growth, respectively, compared with clg-treated mice, the combination therapy of α -erbB2 and α -IL-23 mAbs provided significantly improved suppression of H2N100 tumor growth (Fig. 6A). This combination effect of α -erbB2 and α -IL-23 mAbs was critically dependent upon both NK cells and CD8⁺ T cells (Fig. 6B). Overall, our data demonstrate the potential of neutralizing IL-23 to further improve the therapeutic efficacy of cancer-targeted agents that engage the immune system.

Discussion

Tumor immunosuppression remains one of the major obstacles to many potentially effective cancer therapies and vaccines. In this study, we have shown that a neutralizing mouse mAb to IL-23 alone suppressed early experimental B16F10 melanoma lung metastases and inhibited subcuta-

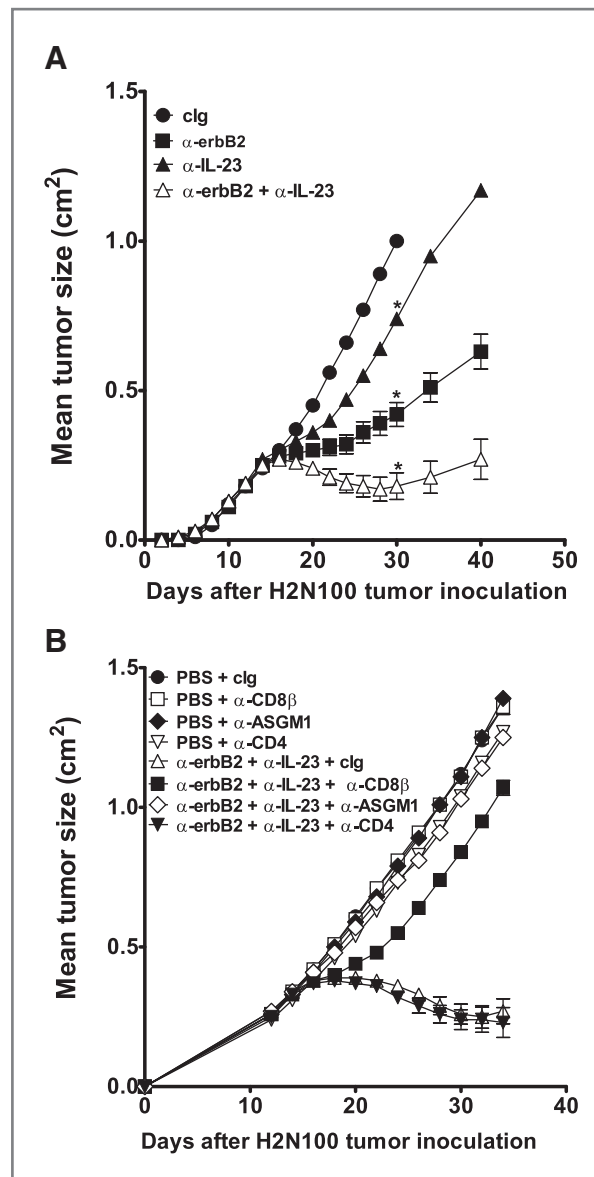


Figure 6. Anti-IL-23 and anti-erbB2 treatment inhibits established H2N100 tumor growth. A, groups of BALB/c WT mice ($n = 5$) were injected subcutaneously with 5×10^5 H2N100 mammary carcinoma cells. Tumor-bearing mice were then treated i.p. with clg, anti-erbB2 (α -erbB2; 50 μ g; days 15, 19, 23, and 27), anti-IL-23 (α -IL-23; days 14–16; 500 μ g), or a combination of α -erbB2 and α -IL-23 mAbs. B, in an experiment similar to A, mice additionally received as indicated 100 μ g of α -CD8 β , α -CD4, or α -ASGM1 i.p. on days 14, 15, 22, and 29 after tumor inoculation. Mice were monitored for tumor growth and data are presented as the mean tumor size (cm^2) \pm SEM. Statistical differences between control Ig-treated mice and α -IL-23 or α -erbB2 alone or α -IL-23- and α -erbB2-treated mice were determined by the Mann-Whitney rank-sum test (*, $P < 0.05$). Data are representative of 2 experiments conducted.

neous growth of EG7 thymoma. These effects were mediated by NK or CD8⁺ T cells, respectively. Furthermore, the use of α -IL-23 mAb in combination with IL-2 or anti-erbB2 mAb treatment significantly inhibited subcutaneous growth of established mammary carcinoma and suppressed established

experimental and spontaneous lung metastases. Notably, Langowski and colleagues did previously describe the anti-tumor activity of a different mouse anti-mouse IL-23 antibody against subcutaneous EP2 mammary cancer cells in mice (8); however, our work represents the first thorough description of the therapeutic potential of an α -IL-23 mAb in cancer.

In humans, IL-23p19 mRNA has previously been shown to be significantly elevated in various human cancers, such as colon, melanoma, lung, breast, and ovarian (8). More recently, IL-23 protein levels were found to be significantly elevated in sera of patients with colorectal (16, 17) and squamous cell carcinomas (18, 19), as well as in the bone marrow of multiple myeloma patients (20). In addition to inducing the hallmarks of chronic inflammation such as metalloproteases, angiogenesis, and macrophage infiltration, IL-23 has also been shown to reduce antitumor immunosurveillance of CD8⁺ and NK cells (8, 9). It is now documented that many tumors are commonly infiltrated and recognized by T cells (7); however, these cells are not able to mediate their effector function at the tumor site due to its immunosuppressive microenvironment (4, 21). Given that anti-IL-23 mAbs are currently in clinical trials for the treatment of psoriasis (Clinical Trials Identifier: NCT01225731), we should soon be able to evaluate the therapeutic potential of neutralizing IL-23 in these patients. Most interesting will be the long-term impact of treating patients and their potential development of malignancies, with the prediction that these patients may be protected against metastasis and tumor development.

Given that chronic inflammatory diseases have been implicated in the induction of many cancers, α -IL-23 mAb therapy may be seriously considered for use in a tumor-preventative setting. For example, people who suffer from inflammatory bowel disease (IBD) or Crohn's disease are at an increased risk of developing colon cancer (22). The observation that IBD is associated with increased expression of IL-23 (23, 24) and polymorphisms within the *IL-23R* gene locus are linked to susceptibility to Crohn's disease (25) provides impetus for treating these patients with anti-IL-23 mAbs. By neutralizing IL-23, the protumorigenic inflammatory environment in the patient may resolve, therefore decreasing their chances of developing cancer. A phase I trial of α -IL-23 mAb will soon commence for the treatment of patients with Crohn's disease, as in psoriasis patients; it will be interesting to monitor these patients for malignancies.

While we and others have clearly shown that host IL-23 plays a major role in promoting tumor initiation, growth, and metastases, it remains unclear as to how exactly IL-23 is mediating its suppressive effect. IL-23 has generally been associated with the development and function of Th17 cells, which is characterized by their ability to produce the proinflammatory cytokines IL-17A, IL-17F, IL-22, and TNF α (2, 26). However, herein and previously (9) our studies have clearly shown that IL-23 mediated its effects independent of IL-17A in these tumor models. With the potential availability of mAbs to neutralize mouse IL-17F, IL-22, and TNF α , the role of these cytokines can be explored in the future. Some studies have attempted to understand the role of IL-23 by administration of the recombinant IL-23 ligand (27). The immunologic and

antitumor effects of IL-23, in gp100 peptide vaccine therapy of established murine melanoma, were tested and neither systemic nor local IL-23 alone had any impact on tumor growth or tumor-specific T-cell numbers. Upon specific vaccination, however, systemic IL-23 greatly increased the relative and absolute numbers of vaccine-induced CD8⁺ T cells and enhanced their effector function at the tumor site. From our studies, it is likely that host IL-23 may be having the opposite effect, cautioning any conclusions regarding mechanism of IL-23 based on ligand administration. Studies in gene-targeted mice presented herein strongly suggest that anti-IL-23 requires host perforin, IL-12, and IFN- γ for full therapeutic effect, illustrating the opposing roles of host IL-23 and IL-12 in these tumor models. In at least 1 tumor model, α -IL-23 enhanced NK cell cytotoxicity at the site of metastases (lung), particularly in combination with IL-2, but did not seem to significantly modulate NK cell proportion or number. Our ability to further define the mechanism by which α -IL-23 suppresses tumors has been limited by the lack of available reagents in the mouse for reliably detecting IL-23 and IL-23R by flow cytometry. Using an IL-23R-GFP (green fluorescent protein) reporter mouse strain, it has been suggested that small populations of CD11b⁺ myeloid cells, CD11c⁺ dendritic cells (DC), and $\gamma\delta$ T cells all constitutively express IL-23R whereas it was not observed on CD8⁺ T cells, B cells, or NK cells (28). However, these observations were only determined in naive mice and it will now be interesting to assess the expression of IL-23R on immune leukocytes in tumor-bearing mice.

Although macrophages and DCs are thought to be the predominant producers of IL-23 (29), a question remains as to whether tumors themselves can secrete IL-23. To date, there have been no convincing studies showing that mouse tumor cell lines or those derived *ex vivo* can produce IL-23. Generally, most studies on IL-23 have used reverse transcriptase PCR as a method to quantitate this cytokine, given the lack of current reagents that can robustly detect IL-23 protein levels. Similarly, in humans, there has been a paucity of studies that have clearly shown that IL-23 can be secreted by human cancer cells, although the reagents for detecting it are more robust. Very recently, an interesting study by Kesselring and colleagues reported that they could detect IL-23 directly in 99% of all *ex vivo* derived head and neck squamous cell carcinoma (HNSCC) tumors (by flow cytometry), although the actual staining data were not shown (18). The authors also reported that they could detect IL-23 in the supernatant of HNSCC cell lines and freshly derived HNSCC tumors; however, these data were also not shown. It remains for these findings to be validated and, if true, other cancer types should also be assessed for their ability to produce IL-23.

From a therapeutic standpoint, our data support the adjuvant use of α -IL-23 in combination with trastuzumab for the treatment of trastuzumab-refractory breast cancer. A number of treatments are being offered to women who are failing trastuzumab treatment; many of them targeting the HER family of receptors (30). Yet, in addition to a landmark study by Clynes and colleagues (31), a recent study by Park and colleagues has also now shown that the mechanisms of tumor

regression of anti-HER2/neu therapy required the activation of innate immunity and CD8 α cells (14). Thus, there is now a strong rationale to combine trastuzumab with agents, such as α -IL-23, that promote T- and NK-cell antitumor immunity. Similarly, IL-2 has been used in the treatment of malignant melanoma, renal cell carcinoma, and some other human malignancies, with some efficacy but limiting toxic side effects (32). It is possible the utility of IL-2 might be improved against metastatic disease at lower doses in combination with α -IL-23 mAbs. IL-23 neutralization may also be considered in combination with certain chemotherapeutic agents such as imatinib mesylate and anthracyclines that are known to engage innate and/or adaptive immunity (33). For example, it has been shown that the therapeutic responses in patients with gastrointestinal sarcoma treated with imatinib mesylate (Glivec) correlated with the induction of IFN- γ production by NK cells (34). Potentially, combining this chemotherapy with IL-23 neutralization may allow for better activation of NK cells, although it remains to be determined whether IL-23 is immunosuppressive in this clinical setting. From cancer surveillance (8, 9) and therapeutic studies herein, it seems that blockade of IL-23 at the point of tumor initiation is far more effective than when tumors are established. These observations alone suggest that IL-23 may be important in suppressing the earliest tumor-specific immune activation while having relatively little impact on restoring anergic T-cell functions in established tumors. In this sense, combinations of α -IL-23 with α -PD-L1

or α -CTLA-4 may also be attractive, acting at different points in the immune response to tumor. Further pursuit of the mechanism of action of IL-23 in tumors and the therapeutic potential of this pathway will provide an important additional armamentarium for cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436–44.
- Martin-Orozco N, Dong C. The IL-17/IL-23 axis of inflammation in cancer: friend or foe? *Curr Opin Investig Drugs* 2009;10:543–9.
- Frey AB, Monu N. Signaling defects in anti-tumor T cells. *Immunol Rev* 2008;222:192–205.
- Rosenberg SA. Overcoming obstacles to the effective immunotherapy of human cancer. *Proc Natl Acad Sci U S A* 2008;105:12643–4.
- Bai A, Higham E, Eisen HN, Wittrup KD, Chen J. Rapid tolerization of virus-activated tumor-specific CD8⁺ T cells in prostate tumors of TRAMP mice. *Proc Natl Acad Sci U S A* 2008;105:13003–8.
- Rosenberg SA, Sherry RM, Morton KE, Scharfman WJ, Yang JC, Topalian SL, et al. Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8⁺ T cells in patients with melanoma. *J Immunol* 2005;175:6169–76.
- Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007;25:267–96.
- Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, et al. IL-23 promotes tumour incidence and growth. *Nature* 2006;442:461–5.
- Teng MW, Andrews DM, McLaughlin N, von Scheidt B, Ngiew SF, Moller A, et al. IL-23 suppresses innate immune response independently of IL-17A during carcinogenesis and metastasis. *Proc Natl Acad Sci U S A* 2010;107:8328–33.
- Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009;27:485–517.
- Ngiew SF, Smyth MJ, Teng MW. Does IL-17 suppress tumor growth? *Blood* 2010;115:2554–5; author reply 6–7.
- Smyth MJ, Thia KY, Cretney E, Kelly JM, Snook MB, Forbes CA, et al. Perforin is a major contributor to NK cell control of tumor metastasis. *J Immunol* 1999;162:6658–62.
- Sondergaard H, Coquet JM, Uldrich AP, McLaughlin N, Godfrey DI, Sivakumar PV, et al. Endogenous IL-21 restricts CD8⁺ T cell expansion and is not required for tumor immunity. *J Immunol* 2009;183:7326–36.
- name>Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell* 2010;18:160–70.
- Stagg J, Sharkey J, Pommey S, Young R, Takeda K, Yagita H, et al. Antibodies targeted to TRAIL receptor-2 and ErbB-2 synergize *in vivo* and induce an antitumor immune response. *Proc Natl Acad Sci U S A* 2008;105:16254–9.
- Stanilov N, Miteva L, Deliyevsky T, Jovchev J, Stanilova S. Advanced colorectal cancer is associated with enhanced IL-23 and IL-10 serum levels. *Lab Med* 2010;41:159–63.
- Ljujic B, Radosavljevic G, Jovanovic I, Pavlovic S, Zdravkovic N, Milovanovic M, et al. Elevated serum level of IL-23 correlates with expression of VEGF in human colorectal carcinoma. *Arch Med Res* 2010;41:182–9.
- Kesselring R, Thiel A, Pries R, Trenkle T, Wollenberg B. Human Th17 cells can be induced through head and neck cancer and have a functional impact on HNSCC development. *Br J Cancer* 2010;103:1245–54.
- Fukuda M, Ehara M, Suzuki S, Ohmori Y, Sakashita H. IL-23 promotes growth and proliferation in human squamous cell carcinoma of the oral cavity. *Int J Oncol* 2010;36:1355–65.
- Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W, et al. Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma. *Blood* 2010;115:5385–92.
- Kim PS, Ahmed R. Features of responding T cells in cancer and chronic infection. *Curr Opin Immunol* 2010;22:223–30.
- Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene* 2010;29:1093–102.

23. Ahern PP, Schiering C, Buonocore S, McGeachy MJ, Cua DJ, Maloy KJ, et al. Interleukin-23 drives intestinal inflammation through direct activity on T cells. *Immunity* 2010;33:279–88.
24. Ahern PP, Izcue A, Maloy KJ, Powrie F. The interleukin-23 axis in intestinal inflammation. *Immunol Rev* 2008;226:147–59.
25. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;314:1461–3.
26. Korn T, Betelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annu Rev Immunol* . 2009;27:485–517.
27. Ustekinumab: new drug. Suspicion of carcinogenicity: too great a risk for psoriasis patients. *Prescrire Int* 2009;18:202–4.
28. Awasthi A, Riol-Blanco L, Jager A, Korn T, Pot C, Galileos G, et al. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol* 2009;182: 5904–8.
29. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat Rev Immunol* 2005;5:521–31.
30. Baselga J. Treatment of HER2-overexpressing breast cancer. *Ann Oncol* 2010;21Suppl 7:vii36–40.
31. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. *Nat Med* 2000;6:443–6.
32. Rosenberg SA. Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. *Cancer J Sci Am* 2000;6Suppl 1:S2–7.
33. Ma Y, Kepp O, Ghiringhelli F, Apetoh L, Aymeric L, Locher C, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Semin Immunol* 2010;22:113–24.
34. Menard C, Blay JY, Borg C, Michiels S, Ghiringhelli F, Robert C, et al. Natural killer cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. *Cancer Res* 2009;69:3563–9.

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Anti-IL-23 Monoclonal Antibody Synergizes in Combination with Targeted Therapies or IL-2 to Suppress Tumor Growth and Metastases

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