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TITLE: Enhancement of Dendritic Cell-Based Immunotherapy Using a Small Molecule TGF-beta Receptor Type I Kinase Inhibitor

PRINCIPAL INVESTIGATOR: Matthew Rausch

CONTRACTING ORGANIZATION: University of Arizona
Tucson, AZ 85722

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Enhancement of Dendritic Cell-Based Immunotherapy Using a Small Molecule TGF-beta Receptor Type I Kinase Inhibitor

Email: rauschm@email.arizona.edu

University of Arizona
Tucson, AZ 85722

Dendritic cells (DC) have become particularly attractive candidates for cancer immunotherapy due to their potent ability to stimulate antigen specific T cells responses. To date DC-based immunotherapy has demonstrated only limited clinical success in the treatment of established tumors. The limited clinical efficacy of existing DC-based cancer vaccines has been attributed in part to suppressive factors produced by the growing tumor, such as transforming growth factor-beta (TGF-β) that has been shown to impair the immunostimulatory capacity of DCs. Therefore, strategies to neutralize the deleterious effects of TGF-β may lead to more effective DC-based cancer therapies. SM16 is a potent small molecule TGF-β receptor type I (TβRI) kinase inhibitor that binds to the ATP-binding pocket of this receptor. Here we show that SM16 inhibits the growth and metastasis of established 4T1 mammary tumors when delivered either via daily i.p. injection or orally through rodent chow. Our data indicate that the anti-tumor effects of oral SM16 were superior to those induced by i.p. injection. In addition, we demonstrate that the anti-tumor efficacy of SM16 is dependent on cellular immunity. DC vaccination failed to improve the efficacy of i.p. SM16, but DC vaccines combined with orally administered SM16 increased primary tumor regression. Furthermore, DC+SM16 therapy enhanced T-cell infiltration into the primary tumor and splenocytes isolated from mice on the combination therapy displayed enhanced IFN-γ production and anti-tumor CTL activity.
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Key Accomplishments

1. SM16 inhibits TGF-β-induced 4T1 tumor cell invasion in vitro.
2. i.p. SM16 has no effect against 10-day established 4T1 tumors.
3. i.p. SM16 at a dose of 40mg/kg inhibits 4T1 pulmonary metastasis formation.
4. 40mg/kg i.p. SM16 inhibits the growth rate of 7-day established 4T1 tumors.
5. DC vaccination does not improve the efficacy of i.p. SM16 in the treatment of 4T1 tumors.
6. Orally delivered SM16 inhibits Smad2 phosphorylation in established primary 4T1 tumors.
7. Oral administration of SM16 inhibits primary and metastatic 4T1 tumor growth.
8. Oral SM16 has no effect on primary and metastatic 4T1 tumors in immunodeficient mice.
9. Primary tumor regression is enhanced in animals treated with oral SM16 in combination with DC vaccination compared to oral SM16 alone.
10. The combination of DC and oral SM16 induces enhanced anti-tumor immunity.

For a detailed description of key accomplishment refer to the results section.

Reportable Outcomes

Submitted Publications:


Presentations:

INTRODUCTION

Dendritic cells (DC) have become particularly attractive candidates for cancer immunotherapy due to their potent ability to stimulate antigen specific T cells responses. A number of pre-clinical and clinical studies using tumor antigen-pulsed DCs to treat a variety of malignancies have demonstrated that DC vaccines can elicit measurable cellular anti-tumor immunity. However, despite these encouraging results, DC-based immunotherapy has demonstrated only marginal clinical success in the treatment of established tumors in cancer patients. These limitations provide rationale for investigating new strategies to augment the efficacy of existing DC-based cancer vaccines. The limited clinical efficacy of DC-based cancer vaccines has been attributed in part to suppressive factors produced by the growing tumor, such as transforming growth factor-beta (TGF-β). Therefore, strategies to neutralize the deleterious effects of TGF-β may lead to more effective DC-based cancer therapies. SM16 is one of a new class of small molecule TGF-β signaling antagonists that block TGF-β signaling by selectively inhibiting the kinase activity of the TGF-β receptor type I (TβRI/ALK5) (1-2). The goal of this study is to use the small molecule ALK5 kinase inhibitor SM16 to enhance the effectiveness of DC-based cancer vaccines in the treatment of established and metastatic TGF-β-producing murine mammary tumors. The hypothesis to be tested is that SM16 therapy will enhance the efficacy of DC vaccines in the treatment of murine mammary tumors by rendering DCs resistant to TGF-β-mediated immunosuppression. The specific aims of this study are to: 1) Determine the effect of SM16 on spontaneous tumor metastasis, 2) Evaluate the effect of the combination SM16 plus DC vaccination on the treatment of primary and metastatic breast cancer, 3) Evaluate the role of immune effector cells in the anti-tumor response following combination therapy with SM16 and DC vaccines.

RESULTS

Aim #1: Evaluate the ability of SM16 to suppress the formation of spontaneous tumor metastasis

Effect of SM16 on 4T1 tumor cell invasion in vitro.

Previous work in our laboratory demonstrated that TGF-β plays an important role in migration and invasion of 4T1 tumor cells (3). In order to test the ability of SM16 to block TGF-β-mediated invasion, 4T1 cells were treated with the drug and assessed for their ability to invade Matrigel in response to exogenous TGF-β. As shown in Figure 1, TGF-β stimulated the ability of 4T1 cells to invade Matrigel nearly 3-fold. This effect was completely inhibited by treatment with 5μM SM16 (P<0.001) (Figure 1).

Figure 1. Effect of SM16 on 4T1 invasion in vitro. 4T1 cells were plated in growth factor-reduced Matrigel invasion cell culture inserts for 24 hr in the presence of 2 ng/ml TGF-β1 with or without 5μM SM16. Invading cells were stained with the DiffQuick staining kit and counted visually at 200X magnification. Data represent mean ± SEM of invading cells in 9 random fields of view. Data are representative of two independent experiments.
Effect of i.p. SM16 on primary and metastatic 4T1 tumor growth.

In order to determine the optimal dose for SM16 therapy, mice bearing 10-day established 4T1 tumors were treated with various concentrations of SM16 via daily i.p. injection for 18 consecutive days. Controls received 18 daily injections of cyclodextran (vehicle). Tumor growth was monitored and lungs were assessed for metastases 28 days post-tumor injection. The data show that SM16 had no significant effect on primary tumor growth at all concentrations tested (Figure 2A). However, SM16 at a dose of 40mg/kg dramatically inhibited the formation of pulmonary 4T1 metastases compared to vehicle-treated controls (P<0.05) (Figure 2B). Animals treated with 40mg/kg of SM16 had an average of 31±13 pulmonary metastatic nodules compared to an average of 121±24 nodules in vehicle-treated controls representing nearly a fourfold reduction in the number of lung metastases. Based on these findings the 40mg/kg dose was chosen for all future experiments.

Figure 2. Effect of i.p.SM16 treatment on primary and metastatic 4T1 tumors. Six-week-old female Balb/c mice were injected s.c. on Day 0 with 5x10⁴ 4T1 cells into the mammary fat pad. On Day 10 the mice were injected i.p. with 10, 20, or 40 mg/kg of SM16. SM16 injections were continued daily until day 28 for a total of 18 injections. Control animals received cyclodextran (vehicle). Lungs were collected at the end of the study, perfused with India ink, fixed in Fekete’s Solution, and surface lung metastases were counted visually. The data represent: A. Mean tumor volume ± SEM of 10 individual mice and B. the number of pulmonary nodules.

Effect of oral SM16 on Smad2 phosphorylation in established primary 4T1 tumors.

A recent report by Suzuki et al. demonstrated that SM16 could be safely and effectively delivered orally through rodent chow to treat a murine model of mesothelioma (2). In addition, they found that the anti-tumor efficacy of p.o. dosing was superior to that of i.p. dosing. Since animals eat periodically over the course of a day, we hypothesized that oral administration of SM16 through rodent chow would allow us to achieve more continuous SM16 dosing that would result in more consistent serum levels of the drug in the animals than once daily i.p. administration, ultimately resulting in more sustained and complete inhibition of TGF-β signaling. In order to determine if SM16 can block TGF-β signaling when delivered orally in the 4T1 model, mice bearing 21-day established 4T1 tumors were fed chow containing 0.45mg SM16/g food for 24hr. and then their primary tumors were analyzed for phosphorylated Smad2 by western blot. The data show that tumors from SM16-treated animals had significantly reduced levels of phosphorylated Smad2 compared to tumors from animals on the control diet (Figure 3).
Figure 3. Orally delivered SM16 inhibits Smad2 phosphorylation in primary 4T1 tumors. Mice bearing 10-day established 4T1 tumors were transferred to either control diet or diet containing 0.45mg SM16/g food for 24 hr. Tumors were homogenized, proteins were resolved by 10% SDS-PAGE, and electrotransferred to PVDF membrane. pSmad2 was detected by incubating the membrane in rabbit anti-human pSmad2 antibody followed by HRP-conjugated anti-rabbit antibody. The membranes were then stripped and incubated with mouse anti-Smad2/3 antibody and membrane-bound total Smad2/3 was visualized with anti-mouse HRP secondary antibody.

Effect of oral SM16 on primary and metastatic 4T1 tumor growth.

We next wanted to determine the efficacy of oral SM16 against primary and metastatic 4T1 tumor growth. For this purpose, mice bearing 10-day established 4T1 tumors were fed chow containing SM16 at a dose of 0.45mg SM16/g chow and primary and metastatic tumor growth was assessed. Each mouse consumed ~3.5g food per day which is equivalent to a daily dose of ~1.6mg or ~80mg/kg body weight/day. Food consumption and animal weights were comparable in the SM16 diet and control diet groups, demonstrating that there was no preference for either diet and there were no overt signs of toxicity (Figure 4A and B). Analysis of serum from animals on the SM16 diet revealed detectable levels of SM16 by 24 hr (Figure 4C). Serum SM16 levels increased slightly and reached a peak of ~18.6 ± 1.3μM after 36 hours. This peak level was maintained until the study was terminated on day 28 (Figure 4C). As expected animals fed normal chow had no detectable SM16 in their serum.

Figure 4D shows that SM16 significantly inhibits the growth rate of established 4T1 tumors compared to animals receiving normal chow (p<0.0001). In addition, one of nine mice in the SM16 diet group experienced complete regression of its primary tumor. The number of metastatic lung nodules was also significantly reduced in mice receiving the SM16 diet compared to mice receiving normal chow (P<0.0001) (Figure 4E). Interestingly, one out of nine (11%) mice receiving SM16 were completely free of pulmonary metastases (Figure 4E). Furthermore, the anti-tumor effect of oral SM16 is superior to what we have previously observed with once daily i.p. administration of the drug.
Figure 4. Effect of oral SM16 therapy on primary tumors and metastases
Six-week-old female Balb/c mice were injected s.c. on Day 0 with $5 \times 10^4$ 4T1 cells into the mammary fat pad. On Day 10 the mice were transferred to chow formulated with 0.45mg SM16/g chow. Control animals received normal chow. Serum was collected from control and SM16-fed animals after 24 hr, 36 hr, and 28 days on diet and analyzed for SM16 levels. Food and animals were weighed every 4 days. The data represent: A. Average food consumption (g±SEM); B. Average weight of mice (g±SEM); C. Average SM16 serum levels (µM±SEM); D. Average tumor size (mm²±SEM); F. Number of pulmonary nodules (boxed numbers represent mean±SEM).
Oral SM16 has no effect on primary and metastatic 4T1 tumors in immunodeficient mice.

A number of recent reports have indicated that cellular immunity plays an important role in the anti-tumor efficacy of other small molecule ALK5 kinase inhibitors (2,4,5). Therefore, we wanted to determine if the anti-tumor effect of SM16 in the 4T1 tumor model was also immune mediated. For this purpose, SCID mice bearing 9-day established 4T1 tumors were fed either control or SM16 diet and primary tumor growth and pulmonary metastases were assessed. As shown in Figure 5A, there was no significant difference in primary tumor growth between SM16-fed and control animals. The tumors grew faster in these animals than in normal immunocompetent BALB/c mice and the study was terminated at day 21 post-tumor injection when animals became moribund. At this time, the average tumor sizes for SM16-fed and control animals were $174.9 \pm 13.57 \text{ mm}^2$ and $135.1 \pm 15.29 \text{ mm}^2$ respectively. Furthermore, there was no significant difference in the number of pulmonary metastases between animals on the SM16 and control diets (Figure 5B). Mice fed control chow had $259 \pm 26.7$ lung nodules compared to $287.0 \pm 13.0$ for mice fed SM16 chow. Taken together, these findings suggest that cellular immunity is critical for the anti-tumor and anti-metastatic activity of SM16.

Figure 5. Effect of oral SM16 on 4T1 tumors in immunodeficient mice
Six-eight-week-old female C.B17/lcrACC(scid) mice were injected s.c. with $5 \times 10^4$ 4T1 tumor cells into the mammary fat pad. On day 9 of tumor establishment, mice were fed diet containing 0.45g SM16/kg chow. Primary tumor sizes were measured and pulmonary metastases were enumerated visually. The data represent: A. Average tumor size ($\text{mm}^2 \pm \text{SEM}$) and B. Number of pulmonary nodules (boxed numbers represent mean±SEM).

Aim #2: Evaluate the effect of the combination of SM16 and DC vaccination on the treatment of primary and metastatic breast in vivo

Effect of the combination of DC+i.p.SM16 on primary and metastatic 4T1 tumors.

Evidence that small molecule ALK5 kinase inhibitors have the ability to modulate endogenous anti-tumor immunity provides a strong rationale for using these agents in combination with active immunotherapy (2,4,5). Since previous work in our laboratory has demonstrated that tumor-derived TGF-$\beta$ can impair a number of critical DC immunostimulatory functions (6), we next wanted to determine if SM16 could enhance the efficacy of DC vaccines in the treatment of established, TGF-$\beta$-producing 4T1 tumors by inhibiting TGF-$\beta$-mediated immunosuppression. To
this end mice bearing 7-day 4T1 tumors were treated with 40 mg/kg SM16 via daily i.p. injection. Injections were continued until day 28 for a total of 21 injections. Animals in the DC groups were injected s.c. with 1x10^6 4T1 lystae-pulsed, TNF-α-matured bone marrow derived DCs on days 9, 14, and 19 post-tumor injection. Controls received 21 daily injections of Captisol (vehicle). Animals were monitored for primary tumor growth and lung metastases were enumerated at the time of sacrifice. The data show that primary tumors treated with SM16 grew at a significantly slower rate compared to tumors from animals in the control or DC alone groups (Figure 6A). Interestingly, there was no significant difference in primary tumor growth between the SM16 alone and DC+SM16 groups (Figure 6A). In addition, SM16 therapy also significantly inhibited the formation of pulmonary 4T1 metastases compared to vehicle and DC alone treated controls (Figure 6B). However, the number of lung metastases in animals treated with the combination of DCs+SM16 did not differ significantly from animals treated with SM16 alone (Figure 6B). The average number of surface lung metastases in animals from the SM16 and DC+SM16 groups were 8±3.7 and 43±18.8 respectively compared to 185±34.1 for vehicle controls and 137±34.6 for DC alone controls.

**Figure 6. Effect of i.p.SM16 in combination with DCs on primary and metastatic 4T1 tumors.** Six-week-old female Balb/c mice were injected s.c. on Day 0 with 5x10^4 4T1 cells into the mammary fat pad. On Day 7 the mice were injected i.p. with 40 mg/kg of SM16. SM16 injections were continued daily until day 28 for a total of 21 injections. Control animals received cyclodextran (vehicle). Aminals in the DC groups were injected s.c. with 1x10^6 4T1 lysate-pulsed TNF-α-matured bone marrow derived DCs on days 9, 14, and 19 post-tumor injection. Lungs were collected at the end of the study, perfused with India ink, fixed in Fekete’s Solution, and surface lung metastases were counted visually. The data represent: A. Mean tumor volume ± SEM of 10 individual mice and B. the number of pulmonary nodules.

**Effect of the combination of DC+p.o.SM16 on primary and metastatic 4T1 tumors.** Since the combination of DC+i.p.SM16 did not improve the efficacy of i.p.SM16 alone, we decided to test if oral SM16 could improve this combination therapy. Based on our data, oral administration of SM16 is superior to that of once daily i.p. injection in its ability to control tumor growth. To this end mice bearing 10-day established 4T1 tumors were transferred to diet containing 0.45mg SM16/g food. These mice were then injected s.c. with 1x10^6 4T1 tumor lysate-pulsed, TNF-α matured bone marrow derived DCs on days 12, 17, and 22 post tumor injection. Controls received control diet, DCs alone, or SM16 diet alone. Primary tumor growth was measured and pulmonary
metastatic burden was quantified. The data indicate that tumors from the SM16 alone and DC+SM16 groups grew at significantly slower rates (p<0.01) than controls (Figure 7A). Furthermore, the average tumor sizes in animals from the SM16 and DC+SM16 groups were significantly smaller (p<0.05) than those in the control groups (Figure 7B). At the time of sacrifice the average tumor sizes of the SM16 and DC+SM16 groups were 51.6±10.0 mm² and 64.7±25.7 mm² respectively compared to 128.3±10.1 mm² for the control mice and 121.9±10.4 mm² for the DC alone mice (Figure 7B). There was no significant difference in primary tumor growth rate or primary tumor size between the SM16 alone and DC+SM16 combination groups. However, more animals in the combination therapy group had complete regression of their primary tumors than animals in the SM16 alone group (Figure 7C). Five of 13 animals (38%) treated with the combination DC+SM16 underwent complete regression of their primary tumor compared to one of twelve animals (8%) treated with SM16 diet alone (Figure 7C). Animals treated with SM16 and DC+SM16 also had significantly fewer pulmonary metastases than control (P<0.001) or DC alone-treated animals (P<0.01). The average number of metastatic nodule in the SM16 and DC+SM16 groups were 4±1.4 and 9±4.9 respectively compared to 148±21.8 for mice on the control diet and 97±27.7 for mice treated with DC alone (Figure 7D).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Percentage of Mice with Complete Primary Tumor Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 (0/13)</td>
</tr>
<tr>
<td>DC Alone</td>
<td>0 (0/13)</td>
</tr>
<tr>
<td>SM16 Alone</td>
<td>8% (1/12)</td>
</tr>
<tr>
<td>DC+SM16</td>
<td>38% (5/13)</td>
</tr>
</tbody>
</table>

A. B. C. D.
Figure 7. Effect of DC+p.o.SM16 on primary and metastatic 4T1 tumors. Six-week-old female Balb/c mice were injected s.c. on Day 0 with 5x10^4 4T1 cells into the mammary fat pad. On Day 10 the mice were transferred to chow formulated with 0.45g SM16/kg chow. Mice in the DC groups were injected with 1x10^6 4T1 lysate-pulled, TNF-α matured DCs on days 12, 17, and 22 post-tumor injection. Control animals received control chow alone, SM16 chow alone, or DCs alone. Primary tumors were measured and the lung metastases were counted visually. The data represent: A. Average tumor size (mm^2±SEM); B. Individual tumor sizes at the time of sacrifice (mm^2); C. Number of mice with complete primary tumor regression; D. Number of pulmonary nodules. (boxed numbers represent mean±SEM).

Effect of DC+p.o.SM16 on anti-tumor immunity

In order to analyze the effect of DC+SM16 on anti-tumor immune responses, splenocytes from animals in the study described above were collected, restimulated with 4T1 lysate in vitro and analyzed for IFN-γ production and tumor specific cytotoxicity. The data indicate that splenocytes from animals from the combination DC+SM16 group produced significantly more IFN-γ (1705.0±56.5 pg/ml) than those treated with SM16 alone (122.5±4.8 pg/ml), DC alone (76.7±2.5 pg/ml), or controls (18.5±0.7 pg/ml) (Figure 8A). As shown in Figure 8B, the cytolytic activity of splenocytes from mice treated with combination DC+SM16 was significantly enhanced compared to splenocytes taken from all three control groups. This enhanced systemic CTL activity was tumor specific as these effector cells failed to lyse irrelevant 12B1 tumor cells (Figure 8C).

![Graph A](image.png)

**A.** IFN-γ (pg/ml)

- Control
- DC Alone
- SM16
- DC+SM16

*p<0.001

![Graph B](image.png)

**B.** Percent Cytotoxicity

- Control
- SM16
- DC alone
- DC+SM16

*P<0.01

![Graph C](image.png)

**C.** Percent Cytotoxicity

- control
- DC alone
- SM16
- DC+SM16

Figure 8. Effect of DC+p.o.SM16 on tumor-specific T cell immunity. A. Splenocytes pooled from treated mice (3/group) were restimulated in vitro for 6 days with 4T1 tumor lysate and IFN-γ production was assayed by ELISA. Data represent mean ± SEM of triplicate samples; B and C. Pooled splenocytes from treated animals (3/group) were restimulated in vitro for 6 days with 4T1 tumor lysate. Effector cells were mixed with ^51^Cr- labeled 4T1 or 12B1 targets for 6hr. and ^51^Cr release was measured. Data represent mean ± SEM of specific lysis.
Aim #3: Evaluate the role of immune effector cells in the anti-tumor response following combination therapy with SM16 and DC vaccines

Effect of DC+p.o.SM16 on tumor infiltrating immune cells

Tumors from animals in the combination DC+p.o.SM16 study described above were also collected and stained for the presence of infiltrating immune cells. A marked increase in CD3$^+$ and CD4$^+$ cells (>50 positive cells per high power field) were observed in tumors from the DC+SM16 group as compared to tumors from the control (~20 positive cells per HPF), DC alone (~25 positive cells per HPF), and SM16 alone (~25 positive cells per HPF) groups (Figure 9A and B). CD8$^+$ cells were rare in tumors from control animals with slightly increased numbers of CD8$^+$ cells observed in tumors from the DC alone, SM16, and DC+SM16 mice (Figure 9C). No infiltrating NK cells were observed in tumor sections from any of the treatment groups (data not shown).

A. CD3

B. CD4

C. CD8
**Conclusions**

In this study we evaluated the effect of the ALK5 kinase inhibitor SM16 in combination with DC vaccination on primary tumor growth and metastasis formation in the aggressive 4T1 mammary tumor model. Our data shows that when delivered via daily i.p. injection, SM16 has no effect on the growth of 10-day (~12mm²) established primary tumors, but does inhibit the growth of pulmonary metastases at a concentration of 40mg/kg. Interestingly, 40 mg/kg i.p.SM16 significantly inhibited the growth of 7-day (~9mm²) established primary 4T1 tumors as well as lung metastases in our DC combination therapy study. SM16 when delivered i.p. was less effective at treating larger established primary tumors, but still led to a marked reduction in the incidence of spontaneous pulmonary metastases in these mice. This observation suggests that i.p.SM16 therapy may be more effective at treating disseminated disease, where tumor burden is minimal and is in agreement with previous reports by us (3) and others (1,4,5,7) that the impact of TGF-β inhibition on metastasis formation is superior to its effect on established primary tumors.

Having shown that i.p. delivery of SM16 resulted in suppression of primary tumor growth and metastasis, we decided to test the efficacy of orally delivered SM16. Suzuki et al. have previously demonstrated that this route of administration was safe and highly effective in a murine mesothelioma model (2). Our data indicate that oral administration of SM16 was safe and resulted in a rapid accumulation of SM16 in the serum that was maintained for the duration of the study. The suppression of primary tumor growth and metastasis of 10-day established 4T1 tumors by orally delivered SM16 was enhanced compared to i.p. administration suggesting continuous dosing may result in more sustained and complete inhibition of TGF-β signaling which may be more desirable for controlling established primary tumors.

Furthermore, we show that the anti-tumor activity of SM16 is dependent on an immune component. The loss of efficacy of SM16 in immunodeficient SCID mice suggests SM16’s mechanism of action requires adaptive cellular immunity. Although we demonstrate in this study that SM16 blocks invasion of 4T1 tumor cells *in vitro*, this mechanism does not appear to play a major role in the anti-metastatic effects of this drug *in vivo* as SM16 had no effect on the formation of lung metastases in immunodeficient SCID mice. This finding is consistent with that of Ge *et al.* (5), who showed that SD-208 had no effect on the metastasis of R3T mammary tumors in athymic nude mice despite its ability to inhibit R3T invasion *in vitro*. However, these findings are in contrast to a report by Bandyopadhyay *et al.*(28) showing that a small molecule ALK5 kinase inhibitor reduced the ability of human breast tumor (MDA-MB-435) xenografts to form spontaneous pulmonary metastases by blocking the tumor cell autonomous effects of TGF-β. The disparity in these findings may be attributed to the different tumor models (mouse versus human) used in both studies.

In this study we also investigated the efficacy of the combination of SM16 and DC vaccination in the treatment of established 4T1 tumors. Our data indicate that the antitumor effect of DC+i.p.SM16 did not differ from that of i.p. SM16 alone. However, orally delivered SM16 in combination with DC vaccination increased the frequency of complete primary tumor regression in animals compared to oral SM16 alone. This data suggests that continuous SM16 dosing which is achieved through dietary administration may be required to produce sustained inhibition of TGF-β-induced immunosuppression to allow for the generation of effective anti-tumor immunity by our DC vaccine.
In this study the superior anti-tumor efficacy of DC+p.o.SM16 correlated with enhanced IFN-γ production and 4T1-specific cytolytic activity by splenocytes and increased T cell infiltration of primary tumors. These findings are consistent with those of Ge et al. (5) in the same tumor model following administration of the ALK5 kinase inhibitor, SD-208. However, unlike Uhl et al. (4) who also showed an increase in tumor-infiltrating NK cells following i.p. administration of SD-208, tumors from animals receiving SM16 alone or in combination with DCs in our study were devoid of NK cells (data not shown). Taken together, our data indicate that SM16 is a safe and highly effective drug for the treatment of metastatic breast cancer that can be used in combination with immunotherapy to enhance primary tumor regression. Since the dose of SM16 used in our oral therapy studies was effective on its own, studies are currently underway in our laboratory to test if DC vaccination can enhance the efficacy of suboptimal doses of SM16.

References


