Antitumor Effects of Cisplatin Combined with Tecemotide Immunotherapy in a Human MUC1 Transgenic Lung Cancer Mouse Model

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Abstract

The goals of the present study were to define the effects of simultaneous cisplatin/tecemotide therapy on tumor development in a human mucin 1 (MUC1) transgenic lung cancer mouse model and to examine the effects of radiotherapy (RTX) on splenocytes, serum cytokines, and immune response to tecemotide. Two hundred twenty-six human MUC1 transgenic C57BL/6 mice were used in five studies designed to assess (i) serum cytokine and immune responses following four weekly 10-μg doses of tecemotide; (ii) the effects of simultaneous administration of cisplatin (2.5 mg/kg × 2 doses/cycle × 4 cycles) and tecemotide (2 cycles × 8 weekly 10-μg doses/cycle) therapy on tumor development, serum cytokines, and immune response; (iii) the dose–response effects of RTX on lymphocyte counts 16 hours following doses of 2 to 8 Gy; (iv) the time course of lymphocyte recovery from 16 hours to 20 days following 8-Gy RTX; and (v) the effects of simultaneous administration of RTX (8 Gy) and tecemotide on the immune response to tecemotide (four weekly 10-μg doses). Serum cytokines were analyzed by multiplex immunoassay, IFN-γ immune responses by enzyme-linked immunosorbent spot (ELISpot), and lung tumor foci by lung whole mounts. Simultaneous cisplatin/tecemotide therapy resulted in significant and additive reduction in lung tumor foci compared with control mice, with significantly elevated serum IFN-γ levels and specific IFN-γ immune responses observed in both tecemotide and cisplatin–treated mice. Finally, neither cisplatin nor radiation interfered with the immune response to tecemotide. Cancer Immunol Res 2(6): 1–9. © 2014 AACR.

Introduction

Lung cancer is the leading cause of cancer-related fatalities worldwide in men and women, with 1.38 million deaths annually (1). Non–small-cell lung cancer (NSCLC) accounts for 80% to 85% of lung cancers, with a poor prognosis for patients with stage III/IV or recurrent disease (2, 3). The standard first-line therapy for patients with unresectable stage III NSCLC is platinum-based doublet chemotherapy and radiotherapy (RTX) with curative intent. However, improvements in survival with the standard therapy are modest, and the 5-year survival rate is only 15% (4). The limited efficacy of chemotherapy (CRT) for NSCLC has raised interest in the development of immunotherapy as an additional treatment modality to prolong survival. The identification of tumor-associated antigens (TAA) in patients with lung cancer has made immunotherapy an attractive alternative to traditional chemotherapy (5, 6).

Tecemotide [formerly L-BlP25 or Stimuvax (Merck KGaA)] is an antigen-specific cancer immunotherapy designed to target the TAA mucin 1 (MUC1) glycoprotein that is aberrantly overexpressed and often under- or unglycosylated in various epithelial cancers, including those of the lung, breast, and prostate (7, 8). Results from several studies have suggested that epithelial carcinogenesis may be facilitated by MUC1 (9–14). Tecemotide elicits a cellular immune response by using T-cell epitopes from the variable number of tandem repeat region (VNTR) of the MUC1 antigen associated with HLA class I molecules, resulting in the induction of IFN-γ secretion into the serum and of MUC1-specific CTL (15–18).

A randomized phase II clinical trial demonstrated a survival benefit of tecemotide and low toxicity in patients with stage IIIb/IV NSCLC who had undergone primary CRT (19, 20). In clinical trials of tecemotide, a single low i.v. dose (300 mg/m²) to a maximum of 600 mg) of cyclophosphamide is administered 3 days before immunotherapy. It is thought that cyclophosphamide enhances delayed-type hypersensitivity humoral and cellular immune responses by reducing T-suppressor function. Recently, results of the global phase III START trial of tecemotide in stage III NSCLC showed that the primary objective of overall survival (OS) prolongation was not met, while predefined subgroup analyses revealed a clinically meaningful prolongation of OS in patients previously treated with concomitant CRT (21).
Previously, we developed a chemically induced lung tumor model in human MUC1 transgenic (hMUC1.Tg) C57BL/6 mice by administering 10 weekly doses of the chemical carcinogen urethane (18). During the carcinogenesis phase, serum proinflammatory cytokines were distinctive and associated with tumor development and progression. Administering two cycles (eight weekly doses/cycle) of tecemotide separated by 11 weeks induced secretion of the Th1 cytokines interleukin (IL)-12, IL-2, and IFN-γ into the blood, and resulted in a reduction of lung tumor foci in urethane-induced hMUC1.Tg mice. Thus, this preclinical lung tumor model may be useful in determining the effectiveness and tailoring the treatment regimens of tecemotide and CRT or other combination therapies.

In the present study, we sought to determine the effectiveness of tecemotide combined with cisplatin chemotherapy in lung cancer and whether cisplatin treatment would affect the tecemotide-induced specific immune response. We also sought to determine the effects of increasing doses of ionizing radiation on lymphocytes in our lung cancer mouse model and whether combining radiation and tecemotide treatment has any effects on the immune response to tecemotide.

Materials and Methods

Chemicals

Urethane, cyclophosphamide, cisplatin (cis-diammineplatinum(II) dichloride), tertiary amyl alcohol, and 2,2,2-tribromoethanol were purchased from Sigma-Aldrich. Tecemotide, placebo, and enzyme-linked immunosorbent spot (ELISpot) peptides (BP25 and scrambled BP1–424) were provided by Merck Serono Research, Merck KGaA.

Animals

To establish our breeding colony, heterozygous C57BL/6 hMUC1.Tg founder mice were purchased from Mayo Clinic (Scottsdale, AZ). For these studies, a total of 226 mixed-sex MUC1.Tg mice were supplied by our breeding colony maintained by the UC Davis Mouse Biology Program and housed at the UC Davis Center for Laboratory Animal Science vivarium. For all studies, mice were housed in cages of four and maintained in a room with constant temperature and humidity on a 12-hour/12-hour light/dark schedule, with free access to food and water. All animal studies were conducted under a protocol approved by the UC Davis Institutional Animal Care and Use Committee. UC Davis (Davis, CA) is an Association for Assessment and Accreditation of Laboratory Animal Care accredited institution. For all mouse studies, the week number refers to the age of the mice.

Radiation treatment

Immediately before the radiation treatment, all mice were anesthetized using a 1.2% solution of Avertin (tribromoethanol) prepared by dissolving 2,2,2-tribromoethanol in tertiary amyl alcohol. Each animal was injected intraperitoneally (i.p.) with 600 µL of the solution using a 25-gauge needle. Once unconscious (~5 minutes), mice were irradiated at the desired doses described in the studies below. Proper anesthesia was ensured by evaluating the righting and tail pinch reflexes. Before treatment, the anteroposterior-posteroanterior diameter of the mice was determined using calipers. RTX was prescribed to midplane and treatment dosing was calculated to cover the entire thoracic cavity with at least 90% of the prescribed dose. The entire RTX treatment protocol was developed by a board-certified medical physicist and a board-certified radiation oncologist. Anesthetized mice were placed in the prone position on 5-cm solid water at laser isocenter, and the treatment area was covered with 0.5-cm bolus to ensure adequate dose build-up. Local thoracic RTX was delivered using an Elekta linear accelerator (Elekta AB) delivering 9-MeV electrons and incorporating a 3 cm × 3 cm cerrobend electron cutout field to ensure that the entire thoracic cavity was encompassed in the radiation field but sparing as much of the cranial vault and abdominal cavity as possible. Following radiation treatments, all animals were monitored until they had sufficiently recovered from anesthesia.

Multiple cytokine assays

The Mouse Cytokine 20-plex Panel (Invitrogen; cat. no. LMC0006) was used to analyze the cytokine levels (pg/mL) in serum samples from the study evaluating serum cytokine and immune responses to tecemotide. The assays were performed according to the manufacturer's instructions. The concentration of each cytokine was calculated relative to the respective standard curves. For 20-plex analyses, cytokine concentrations were acquired on a BioPlex System using BioPlex software version 5.0 (Bio-Rad). For serum cytokine analyses in all other studies, a custom 6-plex panel was used, which consisted of IL-6, IL-1β, IFN-γ, monocyte chemoattractant protein-1 (MCP-1), TNF-α, and macrophage inflammatory protein-1α (MIP-1α). The 6-plex analyses were performed on a Luminex 100/200 system running xPonent software version 3.1 (Luminex Corporation).

In-house control

To establish the in-house control for these assays, wild-type C57BL/6 mice were challenged with lipopolysaccharide (LPS). Mice were injected i.p. with 200 µg of LPS from Escherichia coli serotype O114:B4 (Sigma-Aldrich) dissolved in 1× PBS. At 4 to 5 hours after injection, mice were euthanized by CO2 asphyxiation. Whole blood was collected by cardiac puncture and placed in a clotting tube for isolation of serum by centrifugation. The serum was then stored in liquid nitrogen and stored at −80°C until analysis by multiplex assays on a Luminex system.

ELISpot assay

The ELISpot assay protocol has been described previously (18). Briefly, to isolate lymphocytes, the splenocytes were layered over lymphocyte separation medium (Lonza) and then centrifuged at 600 × g for 15 minutes. The purified and washed lymphocytes were resuspended in improved minimum essential medium (Invitrogen) containing 10% FBS (HyClone), 50 µg/mL streptomycin, and 50 U/mL penicillin (Invitrogen) before cell number counting and viability assessment using an Auto T4 Colliometer (Nexcelom Bioscience).

Lymphocytes (1 × 10⁶/well) were seeded into a 96-well microplate, which was included in the Mouse IFN-γ ELISpot Kit (R&D Systems), and incubated in triplicate at 37°C overnight with no peptide (medium only), BP25 peptide, or scrambled peptide (BP1–424). All peptides were prepared in culture...
Combination of tecemotide, a previously described immune response on lung tumor development, serum cytokines, and effects of cisplatin/tecemotide combination therapy. Mice received four weekly 10-μg doses of either tecemotide (24 mice) or placebo (7 mice) by s.c. injection (100 μL injection volume) at rotating sites using a 25-gauge needle. Three days before starting immunotherapy or placebo treatment, all mice received a single 100-mg/kg dose of cyclophosphamide by i.p. injection. Vials of lyophilized tecemotide or placebo (300 μg each) were reconstituted in 3 mL sterile 0.9% saline within 24 hours before each dose. A total of 11 mice (8 tecemotide and 3 placebo), 10 mice (8 tecemotide and 2 placebo), and 10 mice (8 tecemotide and 2 placebo) were euthanized by CO2 asphyxiation for the collection of blood at 6, 24, and 48 hours, respectively, following the fourth dose of tecemotide or placebo. Blood was collected by cardiac puncture, placed in clotting tubes, allowed to clot for 30 minutes, and then serum was isolated by centrifugation at 3,500 x g for 10 minutes, and then serum was isolated by centrifugation at 3,500 x g for 10 minutes at 4°C. Serum was flash-frozen in liquid nitrogen and stored at −80°C until analysis by multiplex cytokine assay as described above. For ELISpot analysis, spleens were collected from selected mice 24 and 48 hours following the fourth dose of tecemotide or placebo.

Serum cytokine and immune responses to tecemotide
A total of 31 mixed-sex C57BL/6 hMUC1.Tg mice (9 weeks of age) were used to determine whether serum cytokine and immune responses to tecemotide treatment could be assessed following four doses of tecemotide. Mice received four weekly 10-μg doses of either tecemotide (24 mice) or placebo (7 mice) by s.c. injection (100 μL injection volume) at rotating sites using a 25-gauge needle. Three days before starting immunotherapy or placebo treatment, all mice received a single 100-mg/kg dose of cyclophosphamide by i.p. injection. Vials of lyophilized tecemotide or placebo (300 μg each) were reconstituted in 3 mL sterile 0.9% saline within 24 hours before each dose. A total of 11 mice (8 tecemotide and 3 placebo), 10 mice (8 tecemotide and 2 placebo), and 10 mice (8 tecemotide and 2 placebo) were euthanized by CO2 asphyxiation for the collection of blood at 6, 24, and 48 hours, respectively, following the fourth dose of tecemotide or placebo. Blood was collected by cardiac puncture, placed in clotting tubes, allowed to clot for 30 minutes, and then serum was isolated by centrifugation at 3,500 x g for 10 minutes at 4°C. Serum was flash-frozen in liquid nitrogen and stored at −80°C until analysis by multiplex cytokine assay as described above. For ELISpot analysis, spleens were collected from selected mice 24 and 48 hours following the fourth dose of tecemotide or placebo.

Effects of cisplatin/tecemotide combination therapy on lung tumor development, serum cytokines, and immune response
To determine the effect of cisplatin chemotherapy on serum cytokines and the development of lung cancer when used in combination with tecemotide, a previously described hMUC1.Tg lung cancer mouse model was used (18). A total of 80 male C57BL/6 hMUC1.Tg mice began urethane induction at approximately 6 weeks of age. All mice received 10 weekly doses of urethane (0.75 mg/g) by i.p. injection (100 μL) using a 25-gauge needle. In week 23, approximately 7 weeks after the last dose of urethane, mice were randomized into four treatment groups of 20 mice each such that the average weight in each group was approximately equal; the four groups were control, tecemotide, cisplatin, and tecemotide + cisplatin. In week 24, mice in the tecemotide groups began their first 8-week cycle of treatment, 3 days after receiving a single 100-mg/kg dose of cyclophosphamide by i.p. injection. Tecemotide was prepared as described above and administered weekly (10 μg doses) for 8 weeks. Tecemotide cycle 2 began in week 34, approximately 3 weeks following the end of cycle 1, and continued through the end of the study. We showed previously that an antitumor response to tecemotide requires at least two 8-week cycles (18). Just as in cycle 1, a single 100-mg/kg i.p. injection of cyclophosphamide was administered 3 days before the first dose of tecemotide in cycle 2. Concomitant with tecemotide therapy, mice in the cisplatin groups received four cycles of chemotherapy given 4 weeks apart. Each cisplatin cycle consisted of two 2.5-mg/kg doses given by i.p. injection (100 μL) 48 hours apart. Cisplatin cycles 1 to 4 were administered in weeks 24, 28, 32, and 37, respectively. Before each dose, a fresh solution of cisplatin was prepared in sterile 0.9% saline and used within 1 hour.

At the conclusion of the study in week 41, all but 4 mice in each group were euthanized 48 hours after the last dose of tecemotide in cycle 2 for the collection of blood and lungs. Serum was isolated and stored as described. After blood collection, the lungs were harvested for the preparation of whole mounts. The lungs were first inflated with 10% neutral buffered formalin (Thermo Fisher Scientific Inc.), then separated into the five lobes, placed in tissue cassettes, and fixed in formalin for 48 hours, followed by 70% ethanol. Completed lung whole mounts were blinded and then examined by a pathologist to determine the numbers of tumor foci. The remaining 4 mice in each treatment group received an additional dose of tecemotide 48 hours before being euthanized for the collection of blood, spleens, and lungs. Spleens from these mice were processed for ELISpot analysis.

Lymphocyte dose response to RTX
Fifteen mixed-sex C57BL/6 hMUC1.Tg mice (6 weeks of age) were used to determine the effects of increasing doses of radiation on lymphocyte count and serum cytokines. Mice were divided into five groups of 3 mice each, viz., control, 2-, 4-, 6-, and 8-Gy doses of RTX. Sixteen hours following radiation exposure, all mice were euthanized for the collection of blood and spleens. Serum was isolated and stored, and spleens were collected and processed for lymphocyte counts as described in ELISpot analysis. RTX was performed as described in "Radiation treatment."

Time course of lymphocyte recovery following RTX
The effects of an 8-Gy dose of radiation on lymphocyte counts and serum cytokines were followed from 16 hours to 20 days after exposure to assess the time course of lymphocyte recovery. A total of 40 mixed-sex hMUC1.Tg C57BL/6 mice (7 weeks of age) were assigned to five groups of 8 mice each, viz., 16 hours, 48 hours, 7, 9, and 20 days. Within each group, there were 4 control and 4 radiation-treated mice. All mice assigned to RTX were exposed to an 8-Gy dose of radiation as described above. At the scheduled time points following RTX, all mice within each group were euthanized as described above for the collection of blood and spleens. Serum was isolated and stored, and spleens were processed for lymphocyte counts.

Effects of concurrent RTX on the immune response to tecemotide
To determine the effects of RTX delivered simultaneously with tecemotide on the immune response to tecemotide and on serum cytokines, 60 mixed-sex hMUC1.Tg C57BL/6 mice (~8 weeks of age) were assigned to four groups as follows: control (n = 10), RTX (n = 10), tecemotide (n = 20), and tecemotide + RTX (n = 20). Mice in the tecemotide groups received a total of four weekly 10-μg doses as described above. Three days before starting tecemotide treatment, all mice in...
the tecemotide groups received a single 100-mg/kg dose of cyclophosphamide by i.p. injection. Three days after the third dose of tecemotide, all mice in the RTX groups were exposed to an 8-Gy dose of radiation. Mice were administered the fourth and final dose of tecemotide 48 hours after RTX, and then all mice were euthanized as described for the collection of blood and spleens 48 hours after the last dose of tecemotide. Serum was isolated and stored, and spleens were processed for lymphocyte counts and ELISpot analysis.

**Statistical analyses**

In the study of the effects of cisplatin/tecemotide combination therapy on lung tumor development, serum cytokines, and immune response, a one-way ANOVA with Bonferroni multiple comparisons test was used to analyze serum levels of IFN-γ. The ELISpot data in this study were analyzed using a two-way ANOVA with a Bonferroni multiple comparisons test. To analyze lung tumor foci data, an unpaired, two-tailed Student t test was used. In the study assessing the time course of lymphocyte recovery following RTX, an unpaired, two-tailed Student t test was used to assess differences in lymphocyte cell counts and the multiplex serum IFN-γ data. To assess lymphocyte cell counts and IFN-γ serum cytokine data in the study evaluating the effects of concurrent RTX on the immune response to tecemotide, a one-way ANOVA with a Bonferroni multiple comparisons test was used. For the ELISpot data in this study, a two-way ANOVA with the Bonferroni multiple comparisons test was used. All statistical analyses were performed using GraphPad Prism 5 software version 5. A P ≤ 0.05 was considered significant for all analyses.

**Results**

**Serum cytokine profile of concurrent tecemotide/cisplatin combination therapy**

In our previous study, we showed that tecemotide treatment resulted in a significant antitumor effect following the administration of two 8-week cycles with cyclophosphamide pretreatment in a urethane-induced hMUC1.Tg lung tumor mouse model (18). Here, the effectiveness of cisplatin combined with tecemotide in lung cancer was examined. Before this study, a pilot study was performed by administering four doses of tecemotide to healthy hMUC1.Tg mice and then analyzing the serum cytokines at different time points after the final dose to validate the timing of the serum cytokine and immune responses in relationship with tecemotide dosing. The results showed that serum IFN-γ was increased 6 to 48 hours after tecemotide treatment compared with placebo (Fig. 1A). Statistical significance of these increases could not be determined because of limited numbers of placebo mice (n = 2) at each time point. Therefore, tecemotide was administered 48 hours before each analysis endpoint in the cisplatin/tecemotide combination study design (Fig. 2A). A specific IFN-γ immune response to the MUC1 peptide was observed in the pilot study by ELISpot analysis 48 hours following the fourth dose of tecemotide (Fig. 1B), although this difference was not significant. While the pilot study showed that tecemotide-induced serum cytokine release lasts for at least 48 hours, only a modestly increased level of IFN-γ was observed in the sera of lung tumor–bearing mice after two cycles of tecemotide treatment (Fig. 2B) in the current study, which is consistent with our previous report (18). However, levels of IFN-γ in the tecemotide + cisplatin–treated mice were significantly elevated compared with those with placebo control, tecemotide, or cisplatin-alone treatments (Fig. 2B).

**Specific MUC1 immune response to tecemotide with and without cisplatin treatment**

To determine whether cisplatin treatment would affect the tecemotide-induced specific immune response, lymphocytes from mice in each treatment group in the cisplatin/tecemotide

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**Figure 1.** Serum IFN-γ and immune responses to tecemotide immunotherapy. A, average serum IFN-γ concentrations (±SEM) as assessed by multiplex cytokine assay 6 to 48 hours following the fourth dose of tecemotide in hMUC1.Tg mice (n = 8 for tecemotide and n = 2 for placebo, all time points). B, IFN-γ immune response (average SFC/10⁶ cells ± SEM) 48 hours after the fourth dose of tecemotide in hMUC1.Tg mice as assessed by ELISpot (n = 6). SFC, spot-forming cells. Mice were treated with a total of four weekly s.c. doses (10 µg each) of tecemotide or placebo. Three days before the first dose, all mice received a single 100-mg/kg i.p. dose of cyclophosphamide.
combination study were isolated to perform an IFN-\(\gamma\) ELISpot analysis. As shown in Fig. 2C, lymphocytes from tecemotide-treated mice had a significant and specific IFN-\(\gamma\) immune response to the MUC1 peptide, but not to the scrambled peptide. In addition, tecemotide following cisplatin treatment also induced a significant, MUC1-specific immune response, whereas cisplatin-alone did not induce an immune response (Fig. 2C). The difference between tecemotide-alone and tecemotide + cisplatin was not statistically significant.

Additive tumor-suppressive effects of tecemotide/cisplatin combination treatment

MUC1.Tg mice treated with urethane developed lung adenomas with a tumor incidence rate of 100%. The number of tumor foci was significantly lower in the tecemotide- and cisplatin-treated mice than in the untreated mice (Fig. 3). Interestingly, an additive reduction in the number of lung tumor foci was observed with simultaneous tecemotide and cisplatin treatment compared with the single treatment groups (Fig. 3). Compared with the control group, the additive reduction induced by the tecemotide and cisplatin combination was highly significant (\(P<0.0001\)). The reduction in tumor foci induced by the combination was also statistically significant compared with both tecemotide (\(P<0.01\)) and cisplatin (\(P<0.05\)) alone.

Immune recovery following RTX

Lymphocytes are known to be among the most radiosensitive cells in the body. A limitation of our current study design is that although we focus the radiation dose on the thoracic region, part of the spleen is still irradiated. To determine the dose–response effects of radiation on lymphocytes, cell counts were assessed 16 hours after exposure to increasing doses of radiation. As shown in Fig. 4A, the lowest dose of radiation (2 Gy) did not affect cell numbers, while lymphocyte counts decreased in a dose–response fashion following 4, 6, and 8 Gy of...
radiation (Fig. 4A). These differences, however, did not reach the level of statistical significance. To assess the time course of lymphocyte recovery following RTX, lymphocyte counts from 16 hours to 20 days following exposure to an 8-Gy dose of radiation were evaluated. Lymphocyte counts did not begin to recover until 9 days after exposure (Fig. 4B). Full recovery of lymphocytes was observed 20 days after exposure (Fig. 4B). In addition, serum IFN-γ was significantly increased (Fig. 4C) 16 hours after exposure to radiation at a dose of 8 Gy. This may be the result of a nonspecific effect of ionizing radiation on IFN-γ production, as suggested by previously published studies (22, 23).

Effects of concomitant RTX on the immune response to tecemotide
To determine the effects of simultaneous application of RTX and tecemotide on the immune response to tecemotide and on serum cytokines, 60 mixed-sex hMUC1.Tg C57BL/6 mice (~8 weeks of age) were assigned to four groups as follows: control (n = 10), RTX 8 Gy (n = 10), tecemotide (n = 20), and tecemotide + RTX (n = 20). Consistent with the observation of reduced lymphocyte counts following RTX in the above studies, cell counts in the RTX-treated mice were significantly suppressed (Fig. 5A) 4 days after exposure to a radiation dose of 8 Gy. Serum IFN-γ concentrations were significantly elevated in tecemotide-treated animals 48 hours following the last dose of tecemotide (Fig. 5B). Although IFN-γ levels were reduced in the tecemotide + RTX–treated mice compared with mice treated with tecemotide alone, the difference was not statistically significant. IFN-γ ELISpot analysis showed that RTX treatment had no appreciable effect on the immune response to tecemotide, with both the tecemotide-alone and tecemotide + RTX–treated mice showing a specific and significant immune response to the MUC1 peptide (Fig. 5C).

Discussion
In the present study, a human MUC1 transgenic lung cancer mouse model was used to examine the effectiveness of cisplatin and tecemotide used in combination, and whether
cisplatin or local radiation treatment affects the immune response to tecemotide. In agreement with our previous study (18), two 8-week cycles of tecemotide treatment produced a significant reduction in the number of lung tumor foci. Cisplatin-alone treatment had a comparable effect. As is hypothesized for cyclophosphamide, the effects of chemotherapy on the number and function of regulatory T cells (Treg) may be involved in cisplatin’s antitumor effects. A recent study suggests that some chemotherapeutic regimens, such as docetaxel in metastatic breast cancer and castration-resistant prostate cancer, and the combination of cisplatin and vinorelbine in NSCLC, may suppress Treg activity and increase the ratio of T-effector cells to Tregs, while other therapeutic regimens have mixed effects on Tregs, such as tamoxifen combined with leuprolide in breast cancer, and sunitinib in metastatic renal cell carcinoma (24). Clinically, and in the present study, low-dose cyclophosphamide is administered 3 days before starting each cycle of tecemotide. Cyclophosphamide has been shown to reduce both the number of Tregs (25–29) and their immunosuppressive functionality in mice (28) and humans (26). Preclinical data suggest that cisplatin, administered at full therapeutic doses, has a similar effect on Tregs (30, 31) and may prove useful in combination with immunotherapy. Cisplatin has been shown to alter the phenotype of tumor cells (32–34), resulting in increased sensitivity to CTL. Although Treg and CTL activities were not addressed in this study, we did observe an additive inhibitory effect on lung tumor foci with the combination of cisplatin and tecemotide. The tumor-suppressive effects of tecemotide and the combination of cisplatin and tecemotide were associated with a specific and significant immune response to the MUC1 antigen, which suggests that cisplatin does not interfere with the immune response to tecemotide and vice versa. As expected, a MUC1-specific immune response was not observed with cisplatin-alone, which is consistent with cisplatin having a nonspecific tumor inhibitory effect. Furthermore, the observed additive effect in lung tumor foci following treatment with the combination of tecemotide and cisplatin suggests that tecemotide may improve the chemotherapeutic effects of agents such as cisplatin (35).

The inflammatory responses induced by RTX for cancer can exert dual effects. Although they can promote the growth and metastasis of tumors, they can also activate immune response mechanisms that may limit tumor growth (36). Our results showed that when mice were exposed to radiation, a dose–

days after 8-Gy RTX (48 hours after tecemotide dose 4) in control (n = 10), RTX-alone (n = 10), tecemotide-alone (n = 20), and tecemotide + RTX (n = 20)–treated mice. C, IFN-γ immune response (average SFC/10^6 cells ± SEM) as assessed by ELISpot 48 hours following the final dose of tecemotide in control (n = 3), RTX-alone (n = 2), tecemotide-alone (n = 6), and tecemotide + RTX (n = 5)–treated mice. ** P < 0.01; *** P < 0.001; **** P < 0.0001 versus control and tecemotide-alone (A), scrambled peptide versus MUC1 peptide (C). SFC, spot-forming cells. Mice received four weekly 10-mg s.c. doses of tecemotide. Three days before the first dose of tecemotide, mice were injected i.p. with a single 100-mg/kg dose of cyclophosphamide. Three days after the third dose of tecemotide, mice in radiation groups were exposed to an 8-Gy dose of radiation. Two days after radiation treatment, the final dose of tecemotide was given. Forty-eight hours after the last dose of tecemotide, all mice were euthanized.

Figure 5. Lymphocyte counts and serum IFN-γ and immune responses following RTX/tecemotide combination treatment. Average lymphocyte counts (± SEM; A) and average serum IFN-γ concentrations (± SEM; B) 4
response suppression of lymphocytes lasting up to 9 days was observed at doses up to 8 Gy delivered to the thoracic region. Interestingly, even with lymphocyte suppression, a strong immune response was still observed when radiation was administered simultaneously with tecemotide. Taken together, the results of these studies show that the transgenic lung cancer mouse model may prove useful in determining the most efficacious combinations of RTX, chemotherapy, and tecemotide immunotherapy (37).

Finally, the results of the global phase III START trial of tecemotide in stage III NSCLC were recently made available (21). Although the primary objective of prolonging OS was not met, predefined subgroup analyses revealed that patients previously treated with concomitant CRT received a clinically meaningful prolongation of OS. In contrast, patients who were previously treated with sequential CRT seemed to receive no benefit from tecemotide therapy. In this trial, tecemotide was well tolerated, with no safety concerns identified and no evidence of immune-related, treatment-emergent adverse events. The biologic mechanisms underlying these different treatment outcomes are not presently understood. Although the studies described here were not designed to address these concerns, they demonstrated the suitability of the model for use in studies that will attempt to discern the mechanism(s) responsible for these clinical results.

In conclusion, the results of these studies showed that (i) tecemotide combined with cisplatin caused a modest, yet significant reduction in the number of lung tumor foci; (ii) cisplatin did not interfere with the immune response to tecemotide; and (iii) mice treated with localized concomitant radiation maintained a strong immune response to tecemotide. Although the translational relevance of the observed additive reduction in lung tumor foci is presently unknown, it was important to show that tecemotide did not interfere with the efficacy of cisplatin, which is one of the key first-line agents used in the treatment of NSCLC, and that the immune response to tecemotide is maintained with concomitant radiation treatment. If properly sequenced, the addition of RTX to cisplatin chemotherapy and tecemotide may further increase the CTL-driven antitumor activity.

Disclosure of Potential Conflicts of Interest
M.W. DeGregorio is principal investigator of a research grant received from Merck KGaA, and M. Wolf is an employee of Merck KGaA. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: C.-J. Kao, G.T. Wurz, A.M. Monjazeb, M. Wolf, M.W. DeGregorio
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Acknowledgments
The authors thank Drs. Martin Falk, Christoph Helwig, Andreas Schröder, and Christoph Bogdain for their editorial assistance. The authors also thank Melinda Zmerzlikar and Lauren Wilke at the UC Davis Mouse Biology Program.

Grant Support
This research was supported by a research grant from Merck KGaA, Darmstadt, Germany.

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Received November 19, 2013; revised February 14, 2014; accepted February 27, 2014; published OnlineFirst March 10, 2014.

References


Cancer Immunology Research

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Cancer Immunol Res  Published OnlineFirst March 10, 2014.

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doi:10.1158/2326-6066.CIR-13-0205

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