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 radiation therapy (RTX) on splenocytes, serum cytokines, and immune response to tecemotide. Two hundred twenty-six human MUC1 transgenic mice were used in five studies designed to assess: (1) serum cytokine and immune responses following four weekly 10-µg doses of tecemotide; (2) the effects of simultaneous administration of cisplatin (2.5 mg/kg x 2 doses/cycle x 4 cycles) and tecemotide (2 cycles x 8 weekly 10-µg doses/cycle) therapy on tumor development, serum cytokines and immune response; (3) the dose response effects of RTX on lymphocyte counts 16 hours following doses of 2-8 Gy; (4) the time course of lymphocyte recovery from 16 hours to 20 days following 8 Gy RTX; and (5) the effects of simultaneous administration of RTX (8 Gy) and tecemotide on the immune response to tecemotide (4 weekly 10-µg doses). Serum cytokines were analyzed by multiplex immunoassay, interferon gamma (IFN-γ) immune responses by ELISpot, and lung tumor foci by lung whole mounts. Simultaneous cisplatin/tecemotide therapy resulted in significant and additive reduction in lung tumor foci compared to control mice, with significantly elevated serum IFN-γ levels and specific IFN-γ immune responses observed in both tecemotide and tecemotide + cisplatin treated mice. Finally, neither cisplatin nor radiation interfered with the immune response to tecemotide.
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 80-85% 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 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 chemoradiotherapy (CRT) for NSCLC has raised interest in the development of immunotherapies as an additional treatment modality to prolong survival. The identification of tumor-associated antigens (TAAs) in lung cancer patients has made immunotherapy an attractive alternative to traditional chemotherapy (5, 6).

Tecemotide (formerly L-BLP25 or Stimuvax®) is an antigen-specific cancer immunotherapy designed to target the TAA mucin 1 (MUC1) glycoprotein that is aberrantly overexpressed and often under- or un-glycosylated 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 employing T-cell epitopes from the variable number of tandem repeat region (VNTR) of the MUC1 antigen associated with the HLA class I molecules, resulting in the induction of interferon gamma (IFN-γ) secretion into the serum and of MUC1-specific cytotoxic T-lymphocytes (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 chemoradiotherapy (CRT) (19, 20). In clinical trials of tecemotide, a single, low, intravenous dose (300 mg/m² to a maximum of 600 mg) of cyclophosphamide (CPA) is administered three days prior to immunotherapy. It is thought that CPA 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 pre-defined 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 pro-inflammatory 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 into the blood of the Th1 cytokines IL-12, IL-2 and IFN-γ, 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 studies, 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, tertiary amyl alcohol, and 2,2,2-tribromoethanol were purchased from Sigma-Aldrich (St. Louis, MO). Tecemotide, placebo, and ELISpot peptides (BP25 and scrambled BP1-424) were provided by Merck Serono Research, Merck KGaA (Darmstadt, Germany).

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 12h/12h 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 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 prior to 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 (approximately 5 min), mice were irradiated at the desired doses described in the studies below. Proper anesthesia was ensured by evaluating the righting and tail pinch reflexes. Prior to treatment, the AP:PA diameter of the mice was determined using calipers. Radiotherapy 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 radiotherapy 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 radiotherapy was delivered using an Elekta linear accelerator (Elekta AB, Stockholm, Sweden) delivering 9 MeV electrons and incorporating a 3 cm x 3 cm cerrobend electron cutout field to ensure 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.

Multiplex Cytokine Assays

The Mouse Cytokine 20-plex Panel (Invitrogen; cat. #LMC0006, Carlsbad, CA) was used to analyze the cytokine levels (pg/mL) in serum samples from the Serum Cytokine and Immune Responses to Tecemotide study. The assays were performed according to manufacturer’s instructions. The concentration of each cytokine was calculated relative to respective standard
curves. For 20-plex analyses, cytokine concentrations were acquired on a BioPlex System using BioPlex software version 5.0 (BioRad, Hercules, CA, USA). For serum cytokine analyses in all other studies, a custom 6-plex panel was used, which consisted of interleukin (IL)-6, IL-1β, interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF-α), and macrophage inflammatory protein-1 alpha (MIP-1α). The 6-plex analyses were performed on a Luminex 100/200 system running xPonent software version 3.1 (Luminex Corporation, Austin, TX).

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 LPS from Escherichia coli serotype O114:B4 (Sigma-Aldrich) dissolved in 1X PBS. At 4-5 h post-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 flash frozen 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 (18). Briefly, to isolate lymphocytes, the splenocytes were layered over lymphocyte separation medium (Lonza, Walkersville, MD) and then centrifuged at 600 x g for 15 min. The purified and washed lymphocytes were resuspended in improved minimum essential medium (Invitrogen) containing 10% fetal bovine serum (HyClone, Logan, UT), 50 µg/ml streptomycin and 50 U/ml penicillin (Invitrogen) prior to cell
number counting and viability assessment using an Auto T4 Cellometer (Nexcelom Bioscience, Lawrence, MA).

Lymphocytes (1 x 10^6/well) were seeded into a 96-well microplate, which was included in the mouse IFN-γ ELISpot kit (R&D Systems, Minneapolis, MN), 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 medium containing antibiotics at a final concentration of 5 μg/ml. The ELISpot microplates were then developed according to the manufacturer’s instructions.

**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 subcutaneous (s.c.) injection (100 μl injection volume) at rotating sites using a 25-gauge needle. Three days prior to starting immunotherapy or placebo treatment, all mice received a single 100-mg/kg dose of cyclophosphamide (CPA) by i.p. injection. Vials of lyophilized tecemotide or placebo (300 μg each) were reconstituted in a total of 3 ml sterile 0.9% saline within 24 hours prior to each dose. A total of 11 mice (8 tecemotide, 3 placebo), 10 mice (8 tecemotide, 2 placebo), and 10 mice (8 tecemotide, 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 30 min, and then serum was isolated by centrifugation at
3500 x g for 10 min 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 utilized (18). A total of 80 male C57BL/6 hMUC1.Tg mice began urethane induction at approximately six 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 seven 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 eight-week cycle of treatment, three days after receiving a single 100-mg/kg dose of CPA by i.p. injection. Tecemotide was prepared as described above and administered weekly (10 µg doses) for eight weeks. Tecemotide cycle 2 began in Week 34, approximately three 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 eight-week cycles (18). Just as in cycle 1, a single 100-mg/kg i.p. injection of CPA was administered three 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 four 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 1-4 were administered in Weeks 24, 28, 32, and 37, respectively. Prior to each dose, a fresh solution of cisplatin was prepared in sterile 0.9% saline and used within one hour.

At the conclusion of the study in Week 41, all but four 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., Waltham, MA) 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 four mice in each treatment group received an additional dose of tecemotide 48 hours prior to being euthanized for the collection of blood, spleens, and lungs. Spleens from these mice were processed for ELISpot analysis.

**Lymphocyte Dose Response to Radiation Therapy (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 three mice each: control; 2 Gy; 4 Gy; 6 Gy; and 8 Gy. 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. Radiotherapy was administered as described under 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 eight mice each: 16 hours; 48 hours; 7 days; 9 days; and 20 days. Within each group there were four control and four radiation treated mice. All mice assigned to RTX were exposed to an 8-Gy dose of radiation as described under Radiation Treatment. 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 radiotherapy delivered simultaneously with tecemotide on the immune response to tecemotide and on serum cytokines, 60 mixed sex hMUC1.Tg C57BL/6 mice (approximately 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 prior to starting tecemotide treatment, all mice in the tecemotide groups received a single 100-mg/kg dose of CPA 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 Effects of Cisplatin/Tecemotide Combination Therapy on Lung Tumor Development, Serum Cytokines and Immune Response study, a one-way analysis of variance (ANOVA) with Bonferroni’s multiple comparisons test was used to analyze serum levels of IFN-γ. The ELISpot data in this study was analyzed using a two-way ANOVA with Bonferroni’s multiple comparisons test. To analyze lung tumor foci data, an unpaired, two-tailed Student’s t-test was used. In the Time Course of Lymphocyte Recovery Following RTX study, an unpaired, two-tailed Student’s 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 Effects of Concurrent RTX on the Immune Response to Tecemotide study, a one-way ANOVA with Bonferroni’s multiple comparisons test was used. For the ELISpot data in this study, a two-way ANOVA with Bonferroni’s multiple comparisons test was used. All statistical analyses were performed using GraphPad Prism® 5 software version 5. A p-value ≤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 eight-week cycles with CPA 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. Prior to 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-\(\gamma\) was increased 6 to 48 hours after tecemotide treatment compared to placebo (Figure 1A). Statistical significance of these increases could not be determined due to limited numbers of placebo mice (n=2) at each time point. Therefore, administering tecemotide 48 hours prior to each analysis end point was employed in the cisplatin.tecemotide combination study design (Figure 2A). A specific IFN-\(\gamma\) immune response to the MUC1 peptide was observed in the pilot study by ELISpot analysis 48 hours following the fourth dose of tecemotide (Figure 1B), although this difference was not significant. While the pilot study showed that tecemotide-induced serum cytokine release lasts at least 48 hours, only a modestly increased level of IFN-\(\gamma\) was observed in the sera of lung tumor-bearing mice after two cycles of tecemotide treatment (Figure 2B) in the current study, which is consistent with our previous report (18). However, levels of IFN-\(\gamma\) in the tecemotide plus cisplatin treated mice were significantly elevated compared to those with placebo control, tecemotide, or cisplatin alone treatments (Figure 2B).
In order to determine whether cisplatin treatment would affect the tecemotide-induced specific immune response, lymphocytes from mice in each treatment group in the cisplatin/tecemotide combination study were isolated to perform an IFN-γ ELISpot analysis. As shown in Figure 2C, lymphocytes from tecemotide-treated mice had a significant and specific IFN-γ 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 (Figure 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 compared to the untreated mice (Figure 3). Interestingly, an additive reduction in the number of lung tumor foci was observed with simultaneous tecemotide and cisplatin treatment compared to the single treatment groups (Figure 3). Compared to 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 to both tecemotide (p<0.01) and cisplatin (p<0.05) alone.

Immune Recovery Following Radiotherapy

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 Figure 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 (Figure 4A). These differences, however, did not reach the level of statistical significance. To assess the time course of lymphocyte recovery following radiotherapy lymphocyte counts from 16 hours to 20 days following exposure to a radiation dose of 8 Gy were evaluated. Lymphocyte counts did not begin to recover until 9 days after exposure (Figure 4B). Full recovery of lymphocytes was observed 20 days after exposure (Figure 4B). In addition, serum IFN-γ was significantly increased (Figure 4C) 16 hours after exposure to radiation at a dose of 8 Gy. This may be the result of a non-specific 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 radiotherapy and tecemotide on the immune response to tecemotide and on serum cytokines, 60 mixed sex hMUC1.Tg C57BL/6 mice (approximately 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 (Figure 5A) four 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 (Figure 5B). Although IFN-γ levels were reduced in the tecemotide + RTX treated mice compared to 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 (Figure 5C).

Discussion

In the present studies, 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 eight-week cycles of tecemotide treatment produced a significant reduction in the number of lung tumor foci. Cisplatin alone had a comparable effect. As is hypothesized for CPA, the effects of chemotherapy on the number and function of regulatory T cells (Treg) may be involved in cisplatin’s antitumor effects. A recent publication 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 Treg, while other therapeutic regimens have mixed effects on Treg, 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 CPA is administered three days prior to starting each cycle of tecemotide. CPA has been shown to reduce both the number of Treg (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 Treg (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 non-specific tumor inhibitory effect. Furthermore, the observed additive reduction 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 radiation therapy of cancer can exert dual effects. While 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 response suppression of lymphocytes lasting up to nine 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 radiotherapy, 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 overall survival was not met, pre-defined subgroup analyses revealed that patients previously treated with
concomitant chemoradiotherapy received a clinically meaningful prolongation of overall survival. In contrast, patients who were previously treated with sequential chemoradiotherapy appeared 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 biological mechanisms underlying these different treatment outcomes are not presently understood. While 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: (1) Tecemotide combined with cisplatin caused a modest, yet significant reduction in the number of lung tumor foci; (2) Cisplatin did not interfere with the immune response to tecemotide; and (3) Mice treated with localized concomitant radiation maintained a strong immune response to tecemotide. While 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 radiation therapy to cisplatin chemotherapy and tecemotide may further increase the CTL-driven antitumor activity.

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References


Figure Legends

Figure 1
Serum IFN-γ and immune responses to tecemotide immunotherapy.  (A) Average serum IFN-γ concentrations (± SEM) as assessed by multiplex cytokine assay 6-48 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/10^6 cells ± SEM) 48 hours after the fourth dose of tecemotide in hMUC1.Tg mice as assessed by ELISpot (n=6).  SFC= spot forming cells; SEM= standard error of the mean.  Mice were treated with a total of four weekly s.c. doses (10 µg each) of tecemotide or placebo.  Three days prior to the first dose, all mice received a single 100-mg/kg i.p. dose of CPA.

Figure 2
Serum IFN-γ and immune responses following tecemotide/cisplatin combination treatment in a urethane-induced hMUC1.Tg lung cancer mouse model.  (A) Treatment schema:  seven weeks following urethane induction, tecemotide cycle 1 (Weeks 24-31) was begun, followed three weeks later by tecemotide cycle 2 (Weeks 34-41).  Each cycle consisted of eight weekly 10-µg s.c. doses of tecemotide, and three days prior to each cycle, mice received a single 100-mg/kg i.p. dose of CPA.  During tecemotide therapy, mice received four cycles of cisplatin chemotherapy (Weeks 24, 28, 32, 37), each cycle consisting of two 2.5-mg/kg doses given 48 hours apart.  (B) Average serum IFN-γ concentrations (± SEM) (n=20) and (C) IFN-γ immune response (average SFC/10^6 cells ± SEM) (n=4) 48 hours following the final dose of tecemotide in control-, tecemotide-, cisplatin-, and cisplatin + tecemotide-treated mice as assessed by...
multiplex cytokine assay and ELISpot, respectively. *** indicates p<0.001 vs. all groups (B) and MUC1 peptide vs. scrambled peptide (C); ** indicates p<0.01 for MUC1 peptide vs. scrambled peptide (C). SFC= spot forming cells; SEM= standard error of the mean.

Figure 3
Effect of tecemotide/cisplatin combination treatment on the formation of lung tumor foci in a urethane-induced hMUC1.Tg lung cancer mouse model. Mice in the combination treatment group received two eight-dose cycles of tecemotide concurrent with four cycles of cisplatin chemotherapy. Lung whole mounts were prepared 48 hours following the final dose of tecemotide. Average numbers of lung tumor foci (± SEM) are shown (n=20, all treatment groups). * indicates p<0.05 and **** indicates p<0.0001 compared to control. † and †† indicate p<0.05 and p<0.01, respectively. SEM= standard error of the mean.

Figure 4
Lymphocyte dose response and recovery and serum IFN-γ response following radiation therapy. (A) Average lymphocyte counts (± SEM) 16 hours following exposure to 0 (control), 2, 4, 6, and 8 Gy doses of radiation (n=3, all doses); (B) Average lymphocyte counts (± SEM) 16 hours to 20 days following exposure to an 8-Gy dose of radiation (n=4 control and n=4 RTX treated, all time points); (C) Average serum concentrations of IFN-γ(± SEM) 16 hours to 20 days following exposure to an 8-Gy dose of radiation (n=4 control and n=4 RTX treated, all time points). All mice assigned to RTX groups were treated as described under Radiation Treatment. * p<0.05, *** p<0.001, **** p<0.0001 vs. control. SEM= standard error of the mean.
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) four days after 8 Gy RTX (48 hours post 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/10⁶ 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 (B). **** p<0.0001 vs. control and tecemotide alone (A), scrambled peptide vs. MUC1 peptide (C).

SFC= spot forming cells; SEM= standard error of the mean. Mice received four weekly 10-µg s.c. doses of tecemotide. Three days prior to the first dose of tecemotide, mice were injected i.p. with a single 100-mg/kg dose of CPA. 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 2

A

B

C
Figure 5

A

Lymphocytes (x 10^3)

Control

RTX

Tecemotide

Tecemotide + RTX

B

Concentration (ng/ml)

Control

RTX

Tecemotide

Tecemotide + RTX

C

Average SFC/10^6 Cells

Media

Scrambled Peptide

MUC1 Peptide

Control

RTX

Tecemotide

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

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