IL-13 cytotoxin has potent antitumor activity and synergizes with paclitaxel in a mouse model of oral squamous cell carcinoma

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Interleukin-13 receptor-targeted cytotoxin (IL13-PE38) is highly cytotoxic to certain types of human cancers expressing abundant levels of IL-13Ra2 chain. Although IL13-PE38 is being tested in a Phase III clinical trial in brain tumors, the activity of IL13-PE38 alone or when combined with taxane, a chemotherapeutic drug for oral squamous cell carcinoma (OSCC), has not been investigated. Here, we show that approximately 40% of OSCCs (n = 50) in a tissue array are strongly positive for IL-13Ra2, whereas normal oral mucosa (n = 10) expresses very low or undetectable levels evaluated by immunohistochemistry. IL13-PE38 was highly cytotoxic to OSCC cell lines, but not cytotoxic to normal oral fibroblasts. IL13-PE38 mediated a synergistic antitumor effect with paclitaxel in OSC-19 in vitro and in vivo in the orthotopic OSCC tongue tumor model. Real-time tumor growth was monitored by optical imaging using a Xenogen-IVIS imaging system. Treated animals showed significant (p < 0.05) improvement in survival, which correlated with in vivo imaging of tumor response without evidence of visible toxicity. Gene transfer of IL-13Ra2 in oral cancer cells increased sensitivity of OSCC cell line to IL13-PE38 in vitro. Retrovirus-mediated gene-transfer of IL-13Ra2 in HSC-3 into tongue tumors in vivo dramatically enhanced the antitumor activity of IL13-PE38, providing complete elimination of established tumors and prolonging survival of these animals. These results indicate that IL13-PE38 in combination with paclitaxel acting via different mechanisms may be a potential treatment option for IL-13Ra2 expressing OSCC or for the treatment of non-IL-13Ra2 expressing OSCC combined with gene transfer of IL13Ra2.

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Cancer of the head and neck, including the oral cavity, is the sixth most common cancer in the United States.¹ The most common type of oral cavity cancer is oral squamous cell carcinoma (OSCC). It accounts for 2% to 3% of all cancers in the United States, with approximately 34,000 new cases and 7,500 deaths per year.¹ Thus, OSCC remains a significant public health problem. In particular, SCC of the tongue is among the most common tumors of the head and neck.² Despite considerable advances in surgical techniques, irradiation delivery, and improvement in chemotherapeutic strategies, the 5-year survival rates for patients with OSCC in the past 30 years has remained 50% to 60%.¹ In particular, the 3–5 year survival rate of patients with advanced T3 and T4 stages of OSCC has remained poor (20%–30%). Given this dismal state, alternative therapies such as gene therapy and targeted therapy may give new hope for primary or adjuvant treatment for OSCC.

Cytokines, growth factors, and their receptors play a pivotal role in the regulation or stimulation of cancer progression, neovascularization, immunosurveillance, and metastasis.³⁵ Interleukin-13 (IL-13) is one of the T cell-derived Type 2 cytokines that have been implicated in the above processes.⁶ IL-13 binds to two receptor subunits, IL-13Rsα1 and IL-13Rsα2, and stimulates downstream signaling cascades involved in cell proliferation and cytostatic effect or cell death of some neoplastic cells. IL-13Rsα1 is a low affinity receptor but in association with IL-4Rs chain, it forms a high affinity complex; and IL-13 mediates signal transduction through this complex involving either JAK-STAT or PI3 kinase associated with cell proliferation, cell survival, and gene expression.⁶ IL-13Ra2 chain binds IL-13 with high affinity and internalizes without the involvement of other chains. Although this receptor chain had been thought to be nonsignaling, it has recently been reported that IL-13Ra2 is involved in activation of the API pathway leading to up-regulation of TGFβ-1 production.⁷

The expression of IL-13Ra2 has been shown to be elevated markedly in various malignant tumor cell lines and tissues derived from human malignant glioma, head and neck cancer, Kaposis’s sarcoma, ovarian cancer, and renal cell carcinoma. In contrast, normal cells or tissues derived from uninvolved locations of the tumor show very low or undetectable expression levels.⁸ The extent of expression of IL-13Ra2 in head and neck carcinomas seems to be associated with the development of cancer.⁹ The expression of this receptor has also been related to metastasis of breast cancer to lung.¹⁰ Thus, targeting IL-13Ra2 is a reasonable strategy for treatment of certain cancers.

To target IL-13R, IL-13 cytotoxin (IL13-PE38), which is composed of IL-13 and a mutated form of Pseudomonas exotoxin (PE) has been generated.¹¹ IL13-PE38 is highly cytotoxic to IL-13R-positive cancer cells in vitro and in vivo, and this cytotoxin has been investigated in several Phase I/II clinical trials in patients with glioblastoma.¹² In addition, a Phase III clinical trial was completed recently.¹² We previously demonstrated that not only IL-13Ra2-positive head and neck cancer cell lines but also IL-13Ra2-negative cell lines transfected with IL-13Ra2 become dramatically sensitive to IL13-PE38.¹³ As OSCCs are fairly accessible tumors, a gene transfer approach can be easily applied to expand the therapeutic opportunity for targeting this cancer.

In the current study, we examined the expression of IL-13R in OSCC cell lines and tumors in situ. We explored the antitumor effects of IL-13R targeted cytotoxic with or without gene transfer of IL-13Ra2 followed by IL13-PE38 therapy. In addition, we investigated the potential of IL13-PE38 to enhance the effects of chemotherapeutic agents for oral cancer therapy. Platinum drugs such as cisplatin and carboplatin, combined with Fluorouracil (5-FU), have been recognized as standard chemotherapy for OSCC. Recently, taxanes including paclitaxel and docetaxel, have been used in combination with platinum drugs to improve the prognosis.¹⁷–¹⁹ As taxanes can block cell replication, arrest cells in the G2 and M phase of the cell cycle, and stabilize cytoplasmic microtubules, a different mechanism of action than IL13-PE38, we explored whether IL13-
PE38 synergizes with paclitaxel. We show that simultaneous administration of IL13-PE38 and paclitaxel can exert a synergetic antitumor effect. In addition, we demonstrate that sequential IL-13Ra2 gene transfer followed by IL13-PE38 administration is particularly effective in the murine orthotopic tongue cancer model.

**Material and methods**

**Cell culture and reagents**

Human OSCC cell lines OSC-19, HSC-3, and KON were purchased from Japan Health Sciences Foundation, Health Science Research Resources Bank (Osaka, Japan). SCC-25 cell line was purchased from the American Type Culture Collection (Manassas, VA). KCCT873 cell line was established in the Department of Otolaryngology, Yokohama City University School of Medicine Research Institute, Kanagawa Cancer Center, Yokohama, Japan. Human normal gingival fibroblasts (HGF) cell line was obtained from ScienCell (San Diego, CA). Paclitaxel was obtained from Bristol-Myers Squibb (Tokyo, Japan). Recombinant IL-13 was purchased from PeproTech (Rocky Hill, NJ). Recombinant IL13-PE38 was produced and purified in the laboratory as described previously.

**Tissue array and immunohistochemistry**

A tissue array containing 60 different benign and malignant oral tumors was purchased from a commercial source (Imgenex, San Diego, CA). Immunohistochemistry was performed on all sections using a Vector ABC peroxidase kit according to manufacturer’s instructions (Vector, Burlingame, CA). Sections were incubated with anti-human IL-13Ra2 monoclonal antibody (Diaclone, Besancon, France) or isotype control (IgG). Tissue sections were scored independently by two authors (M. K. and M. H.) on the basis of the intensity of staining: −, negative; +, slightly positive; +, moderately positive; and ++, strongly positive.

**Semi-quantitative RT-PCR**

Total RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA), and RT-PCR performed using specific primers as described previously.

**Protein synthesis inhibition assay**

The in vitro cytotoxic activity of IL13-PE38, paclitaxel, and their combination was measured by the inhibition of protein synthesis. Briefly, cells (1 × 10^4) were cultured in leucine-free medium with varying concentrations of IL13-PE38 and paclitaxel for 24 hours at 37°C in a 96-well plate. One μCi of [3H] leucine (NEN Research Products, Boston, MA) was added to each well and incubated for an additional 4 hours. All assays were performed in quadruplicate, and the concentration of IL13-PE38 at which 50% inhibition of protein synthesis occurred was calculated (IC50).

Drug interaction between IL13-PE38 and paclitaxel was assessed at a concentration ratio of 1:1, using the combination index (CI), where CI < 1, CI = 1, and CI > 1 indicate synergistic, additive, and antagonistic effects, respectively. On the basis of the isobologram analysis for mutually exclusive effects, the CI value was calculated as follows: CI = (D1)/(D1) + (D2)/(D2), where (D1) and (D2) are the concentrations of individual drug IL13-PE38 and paclitaxel, respectively, required to inhibit cell growth by 50%, and (D1) and (D2) are the drug concentrations in combination treatments that also inhibit cell growth by 50% (iso-effective as compared with the single drugs).

**Apoptosis detection by TUNEL staining in vitro**

Cells (0.5 × 10^5) were plated in chamber slide and incubated with medium only (control) or IL13-PE38 (100 ng/mL) for 48 hrs. Cells were then washed and fixed with 1% paraformaldehyde in PBS, and stained with the TUNEL-based apoptosis detection kit (Millipore, Billerica) as per manufacturer’s instructions. Apoptotic cells were assessed and measured by fluorescent microscopy.

In vitro retroviral transduction

Retrovirus-mediated overexpression of IL-13Ra2 or firefly luciferase was performed with the ViraPort Retroviral Gene Expression System (Stratagene, La Jolla, CA) following the manufacturer’s instructions with minor modifications. The full coding region of human IL-13Ra2 cDNA was PCR-amplified and cloned into the retroviral expression vector pFB-Neo to generate pFB-Neo-IL-13Ra2. To obtain retrovirus-containing supernatants, 293T cells were cotransfected with pVPack-VSV-G, pVPack-GP, and pFBneo-IL-13Ra2 or pFB-luciferase. Forty-eight hours after transfection, the medium containing retroviruses was collected, filtered, treated with DEAE-Dextran (1 μg/mL), and transferred to the cells. Infected cells were selected with G418 (700 μg/mL) antibiotic for 10 days. Expression of luciferase was confirmed using the IVIS system (Xenon, Alameda, CA).

**SC xenografted oral tumor model**

All animal experiments were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. OSC-19 (7 × 10^6) were suspended in 150 μL PBS and inoculated subcutaneously (SC) into the right dorsal flank of 6-week-old male BALB/c nude mice. Tumor growth was monitored using Vernier calipers, and tumor size (mm^3) was calculated by multiplying length and width of tumor on a given day. Treatments were started 15 days after tumor cell inoculation, when tumors reached a mean size of ~20 mm^3 (4–5 mm diameters). Mice were divided into different therapeutic groups and one control group (6–7 mice per group). Mice were injected with excipient (0.2% human serum albumin in PBS) or IL13-PE38 by either intraperitoneal (IP; 500 μL using a 27-gauge needle) or intratumoral (IT; 30 μL using a Hamilton microinjection syringe) routes. HSC-3 SC tumors were created by inoculating 5 × 10^5 cells. One week later, suspensions of retrovirus for IL-13Ra2 transduction (5–10 × 10^6 TU/50 μL) were administered by direct IT injection.

**Orthotopic-xenografted tongue tumor model**

For the orthotopic oral tumor model, OSC-19 (2 × 10^5 cells/30 μL) or HSC-3 (3 × 10^5 cells/30 μL) were inoculated into the left edge of the tongue of 6-week-old male nude mice. Treatments were started 5 days after tumor implantation. Mice were randomly divided into different therapeutic groups and one control group (6–7 mice per group). Animals were injected with excipient or IL13-PE38 (50 μg/kg) twice a day for 5 days intraperitoneally or 100 μg/kg on alternate 3 days intratumorally. Paclitaxel was injected intraperitoneally at a dose of 10 mg/kg daily for 5 times a week for 2 weeks.

**Bioluminescence imaging and analysis**

Real-time tumor growth was monitored by optical imaging using a Xenogen-VIS cooled CCD optical system. Mice were anesthetized with 3% isoflurane after IP injection with 75 mg/kg body weight D-Luciferin (Xenogen). Five minutes after injection of the α-Luciferin, images were acquired for 10 sec to 2 min using Living Image analysis and acquisition software (Xenogen). A photographic image taken, onto which the pseudocolor image representing the spatial distribution of photon counts was projected. For bioluminescence imaging (BLI) plots, photon flux was calculated for each mouse by using a square region of interest encompassing the head of the mouse in a supine position. This value was scaled to a comparable background value (from a luciferin-injected mouse with no tumor cells), and then normalized to the value obtained immediately after xenografting (day 0), so that all mice had an arbitrary starting BLI signal of 100.

**Statistical analysis**

The statistical significance of tumor growth was calculated with the unpaired t-test. All statistical tests were two-sided. Survival rates were calculated by the Kaplan-Meier method. For analysis of
statistical significance between the therapeutic group and the control group in survival assays, the log-rank test was used. The difference in IL-13Rα2 expression between malignant and normal oral tissues was calculated by the χ² test.

Results
Expression of IL-13Rα2 chain in oral tissue, OSCC, and cell lines

Tissue sections from 10 normal oral tissues and 50 OSCC samples were analyzed by immunohistochemical analysis for the expression of IL-13Rα2 subunit, which is a predominant IL-13 binding protein. Representative tumor specimens showing high levels of IL-13Rα2 expression (sample number B7 and D5) are shown in Figure 1a. In contrast, normal oral cavity tissue specimens showed no staining or weak staining (−, ±) for IL-13Rα2 chain (Fig. 1a, sample number F4 and F10). Approximately 40% of OSCC tissues were strongly positive for IL-13Rα2 chain (++) and 40% moderately positive (+). Although the sample size is small, as shown in Table I, higher grade OSCC tumors tended to show ++ IL-13Rα2 expression compared with lower grade tumors (23% Grade I, 45.5% Grade II, and 50% Grade III show + receptor expression).

We also determined the expression of IL-13Rα2 mRNA by semi-quantitative RT-PCR analysis in 6 OSCC and HGF cell lines. The expression of mRNA for IL-13Rα2 was detected in OSCC cell lines, and PM-RCC, a renal cell carcinoma cell line, served as a positive control, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Cells (1 × 10⁶/well) were cultured in leucine-free medium containing various concentrations of IL-13-PE38 with or without 1 μg/mL of IL-13 for 20–22 hrs. Cells were then pulsed with 1 μCi of [3H]-leucine for 4 hr. The amount of radioactivity incorporated into cells was measured. Detection of apoptosis in OSCC cell lines. OSCC cell lines (OSC-19, SCC-25, HSC-3, and PM-RCC) served as positive controls, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as an internal control.
Synergistic cytotoxicity of IL13-PE38 and paclitaxel in the OSC-19 cell line

Taxanes have recently been used with other reagents as adjuvant or neoadjuvant chemotherapy drugs in patients with OSCC.18,19 We therefore examined the cytotoxicity of paclitaxel, one of the taxanes, in the OSC-19 cell line, which showed sensitivity to IL13-PE38. Paclitaxel alone mediated a concentration-dependent inhibition of protein synthesis with an IC50 of 30 μg/mL (Fig. 2a). However, the combination of paclitaxel with IL-13-PE38 inhibited protein synthesis more effectively than either agent alone (Fig. 2b). The IC50 of IL13-PE38 became 10, 0.23, 0.14, 0.01, 0.001, and 0.0002 ng/mL in combination with 0.0001, 0.001, 0.01, 0.1, 1, and 10 μg/mL paclitaxel, respectively, compared with 30 ng/mL without paclitaxel (Figs. 2b and 2c). The IC50 concentration of drug causing 50% inhibition of protein synthesis) of IL-13-PE38 became 36, 1.51, 0.82, 0.25, 0.12, and 0.0024 ng/mL in combination with 0.0001, 0.001, 0.01, 0.1, 1, and 10 μg/mL paclitaxel, respectively, compared with 36 ng/mL without paclitaxel (Figs. 2b and 2c). In contrast, the IC50 concentration of paclitaxel (Fig. 2b) was 10 μg/mL.

Synergistic cytotoxicity of IL13-PE38 and paclitaxel in the OSC-19 cell line

To investigate the in vivo antitumor effect of IL13-PE38 in OSCC, we first established a subcutaneous human OSC-19 tumor xenograft in nude mice. We injected IL13-PE38 (50 μg/kg × 5 days) IP twice a day or 50 μg/kg on 3 alternate days IT in OSC-19 tumor-bearing mice. The IL13-PE38 treatment started on day 15 when palpable tumors developed. As shown in Figure 3a, IL13-PE38 mediated significant antitumor activity when administered IT, while IP administration of IL13-PE38 with 50 μg/kg dose did not significantly decrease the tumor size compared with control (p < 0.5, Fig. 3b). IL13-PE38 IT treatment on alternate days for 3 days significantly reduced SC tumors in all treated mice (p < 0.04). We next evaluated the combination effect of IL13-PE38 and paclitaxel in the OSC-19 SC tumor model. IL13-PE38 was injected IP or IT at 50 μg/kg for 5 or 3 days, respectively. Paclitaxel was injected IP at a dose of 10 mg/kg 3 times a week for 3 weeks. By day 49, the combination of IP IL13-PE38 and paclitaxel treatment revealed 50% reduction in OSC-19 SC tumor size (Fig. 3b) (p < 0.04). On the other hand, IL13-PE38 IT treatment combined with IP paclitaxel significantly reduced tumor size compared with either paclitaxel or IL13-PE38 alone (p < 0.007 and p < 0.04, respectively). The combination of IT IL13-PE38 with paclitaxel eradicated OSC-19 tumor in all mice (Fig. 3a).

In vivo live animal optical imaging of SCC implanted on tongue

To investigate the in vivo tumor growth in the OSC-19 subcutaneous xenograft model, we injected luciferase-expressing OSCC cell line into the tongue of nude mice for real-time determination of tumor growth. Our transduction technique using retroviral vector revealed consistent expression of luciferase in OSCC cells. There were no significant differences in morphology and growth rates between parent and luciferase-expressing cells (data not shown). Luciferase-transduced OSCC (OSCC-Luc) cells injected into tongues of nude mice formed tumors in all mice and yielded a high rate of regional metastases by week 2–3 (60%–80%, Fig. 4a arrow). Noninvasive quantitative measurements of external visible bioluminescent area (total flux, photons/sec/cm²) enabled the construction of in vivo tumor growth curves, which were remarkably log-linear (Fig. 4b). IL13-PE38 treatment appeared to reduce the rate of tumor growth, though not significantly (p = 0.3). When combined with paclitaxel, suppression of tumor growth was significant (p < 0.05). The reduction of tumor size on day 31 was 47.9% in the paclitaxel group, 74.8% in IL13-PE38 IP treatment, 82.1% in combination of IL13-PE38 IP and paclitaxel, 99.9% in IL13-PE38 IT treatment, and 99.9% in combination of IL13-PE38 IT and paclitaxel group compared with the nontreatment group (Fig. 4b and data not shown).

Synergistic cytotoxicity of IL13-PE38 and paclitaxel in the OSC-19 cell line

We also evaluated the efficacy of IL13-PE38 alone and in combination with paclitaxel on survival of mice with orthotopic OSCC. As shown in Figure 4c, the median survival time of mice was 40 days in the nontreatment group. Although paclitaxel treatment extended the survival of animals (median survival time, 49 days), this difference was not significant (p = 0.12). On the other hand, both IP and IT administration of IL13-PE38 extended survival of tumor-bearing mice significantly (p < 0.003 and p < 0.005, respectively). Although both IT and IP IL13-PE38 treatment combined with paclitaxel significantly prolonged survival, the combination of paclitaxel and IT administration of IL13-PE38 was most efficient. By day 40, 4 of 6 mice showed complete eradication of tumor, and these remained tumor-free through day 120, when experiment was terminated. During these treatments, no visible signs of abnormality or loss in body weight were observed.
in treatment groups. However, mice treated with paclitaxel or untreated control showed loss in body weight despite the weight of growing tumors (Fig. 4).

In vitro IL-13Rx2 gene transfer increases sensitivity of OSCC to IL13-PE38

To investigate the effect of IL-13Rx2 on cytotoxicity of IL13-PE38 to sensitive and non-sensitive OSCC cell lines, retroviral transduction of IL-13Rx2 was performed. OSC-19, HSC-3, and KON cells were incubated with viral supernatant (MOI: 50) and then selected by G418 antibiotic. The sensitivity of IL-13Rx2-infected OSC-19, HSC-3, and KON cell lines to IL13-PE38 was significantly increased compared with control cells. IC_{50}s of 0.008, 100, and 800 ng/mL, respectively, were observed in retro-IL-13Rx2-infected OSC-19, HSC-3, and KON cell lines compared with their control counterparts (IC_{50} of 8, >1000, and >1000 ng/mL) (Fig. 5a). These results indicate that gene transfer of IL-13Rx2 further enhanced cytotoxicity of IL13-PE38 in oral tumor cells.

Combination effect of IL-13Rx2 gene transfer by non-replicating retrovirus IT injection followed by IL13-PE38 treatment

To increase the therapeutic effect of IL13-PE38, we injected replication-defective IL-13Rx2 retrovirus supernatant directly in HSC-3 tumors, which are not sensitive to IL13-PE38. Initially, we used a SC tumor model to determine the combination effect by monitoring the tumor size. Mice bearing HSC-3 SC tumors received IT injection of retro-IL-13Rx2 or retro-luciferase on day 8, 10, and 12. Subsequently, IL13-PE38 (100 μg/kg/day) or PBS was administered IT to the tumors on 3 alternate days, beginning on day 31. From retroviral vector injection (day 8) to initiation of IL-13 cytotoxin treatment (day 31), no significant difference in the
of 6 mice survived until day 120, when the experiment was terminated. IL13-PE38 IT injection after retro-Luc slightly extended survival but no significant difference was observed compared with gene transfer alone.

Discussion

We demonstrate that 40% human OSCC tumors and cell lines express high levels of high-affinity IL-13Rα2, whereas normal oral samples express undetectable or very low levels of IL-13Rα2. These receptor positive tumor samples were sensitive to the cytotoxic effect of IL-13 receptor-directed cytotoxin IL13-PE38, which mediated significant antitumor activity in OSCC tumor cell lines in vivo and in animal models of human oral cancer. These results are consistent with our previous report of expression of IL-13Rα in head and neck tumors and targeting by IL-13-PE, however, that study included only few samples of oral cavity tumors.9,16 In this study, we specifically focused on OSCC tumors. In addition, we show antitumor activity of IL13-PE38 in orthotopic OSCC tumor model and also demonstrate a synergistic effect of taxane with IL13-PE.

OSCC tumor cell lines that did not express IL-13Rα2 showed no sensitivity to IL13-PE38. About 60% of tumor specimens showed positive to weakly positive levels of IL-13Rα2. For those tumors, we demonstrated that gene transfer of IL-13Rα2 dramatically enhanced their sensitivity to IL-13 cytotoxin in vitro. As OSCC is generally located on the surface of the oral cavity, we also performed direct gene transfer of IL-13Rα2 into insensitive tumor followed by IL13-PE38 therapy. A retroviral vector was used for this purpose. As recombinant retrovirus infects only actively proliferating cells, it limits unintended gene expression in normal tissues surrounding tumor since normal mucosa and muscle cells usually divide more slowly than target tumor cells.26,27 Treatment of these animals with IL13-PE38 caused regression of established tumors and significantly improved survival of animals with orthotopic tongue tumors. Retrovirus-mediated gene transfer in vivo not only allowed expression of IL-13Rα2 for a prolonged period, but also demonstrated an expansion of the therapeutic window of IL13-PE38 treatment. Both IL-13Rα2 positive and negative tumors could be targeted by IL13-PE38 after gene transfer.

Paclitaxel, an approved drug, synergized with IL13-PE38 in mediating antitumor activity in vitro and in vivo. This combination therapy caused complete eradication of tumors in 66% of mice and significantly extended survival of mice bearing OSCC orthotopic tumors. We chose suboptimal doses of IL13-PE38 to determine synergistic effect of combination therapy with paclitaxel. However, more aggressive IT treatment with IL13-PE38 without paclitaxel may also be valuable and may negate addition of paclitaxel. However, as higher doses of IL13-PE may cause liver toxicity because of metabolism, lower dosages are preferred. Nevertheless, Phase I clinical trial by IT administration of IL13-PE will be needed to assess safety and activity by this route of administration.

The mechanism of synergistic antitumor effect of IL13-PE and paclitaxel was considered interesting. IL13-PE38 mediates cytotoxic effect by PE portion of the IL13-PE38 molecule. After binding to IL-13R, IL13-PE38 is internalized into the cytosol, and then PE domain III inhibits protein synthesis by ADP-ribosylation of elongation factor 2.12 This leads to cell death by not only necrosis but also by apoptosis of target cells as shown in this study as well. Paclitaxel on the other hand blocks cell replication, arrests cells in the G2/M phase of the cell cycle and cells are unable to form a normal mitotic apparatus.20 These are completely unrelated mechanisms, which may provide a possible molecular basis for synergism. As combination therapy was well tolerated without any visible signs of toxicities, our results indicate that IL13-PE38 and paclitaxel combination therapy may afford better tumor response than standard chemotherapy or single drug treatment. For more than a decade, the combination of platinum (including cisplatin

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**FIGURE 3** – Antitumor activity of IL13-PE38 and paclitaxel against OSCC-19 SC tumors. Female nude mice bearing subcutaneous OSCC-19 tumor were treated with either IL13-PE38 at 50 μg/kg/day of cytotoxin IT on days 15, 17, and 19 (once a day) (a) or 50 μg/kg twice a day IP from day 15 to day 19 (b) with or without paclitaxel (10 mg/kg) on days 15–19 and 22–26, respectively. Control indicates the group in which mice were treated with PBS only. **CR**, complete response; and * not significant, ** p < 0.05.**
and carboplatin) and 5-fluorouracil (5-FU) has been a standard of care for management of the patients with advanced or recurrent OSCC. Taxanes, including paclitaxel and docetaxel, are now widely being accepted as a new first-line chemotherapy combined with these drugs in advanced ovarian and HNSCC. Several clinical trials combining paclitaxel with molecular target therapy have been initiated. In addition, emerging evidence suggests the advantage of paclitaxel in the treatment of OSCC. The regional effectiveness of radiation can be enhanced by the accompanying radiosensitizing chemotherapy, including taxanes. Thus, our results indicate that paclitaxel may synergize with IL13-PE38 in mediating antitumor activity in clinical trials as well.

For clinical application of IL13-PE38, several considerations in terms of feasibility of delivery and toxicity are of paramount importance. Because OSCC is a loco-regional disease, IT injection of IL13-PE38 is an ideal approach. We previously demonstrated that a significant level of IL13-PE38 could be achieved at the tumor site for a longer period when injected directly into tumors without flowing into blood circulation. Due to this feature, very high doses of IL13-PE38 (up to 750 μg/kg) from day 8 on three alternate days with paclitaxel (10 mg/kg/day, on day 8–12 and day 15–19). Arrow indicates lymph node metastasis. Tumor growth is indicated as bioluminescence imaging plots. Photon flux was calculated for each mouse by using a square region of interest encompassing the head of the mouse. This value was scaled to a comparable background value and then normalized to the value obtained immediately after xenografting. Kaplan-Meier survival curves of mice treated with vehicle alone, paclitaxel, or IL13-PE38 (IP or IT injection), with or without paclitaxel. Average body weight of mice in each group was measured at least once a week. Control indicates the group in which mice were treated with PBS only.

As there is ample data available that show safety, tolerability, and activity of IL13-PE38, additional preclinical studies with IL13-PE38 may not be needed before conducting a clinical trial, but when combined with paclitaxel additional pharmacology and toxicology studies may be needed. In addition, for gene transfer of IL-13Ra2 followed by IL13-PE38 administration, additional preclinical studies will also be needed before beginning a Phase I clinical trial. Retrovirus mediated gene transfer technology has evolved over the last 15 years and therefore, it is feasible to begin clinical trial with this approach without significant challenges. Since in our model, retroviral mediated gene transfer of IL-13Ra2 followed by IL13-PE38 therapy significantly improved antitumor activity without any visible toxicity, it is reasonable to propose clinical testing of this approach for OSCC therapy.

As PE is a component of IL13-PE38 and a foreign bacterial protein, immune response to PE would be a problem when a prolonged repeat administration of IL13-PE38 is needed. However, as only 3 injections of IL13-PE38 on alternate days in 1 week were sufficient to cause a profound or a curative antitumor response in animal model, we may not need repeat administration and, thus, avoid immunity issue. In addition, we have previously demonstrated that significant levels of IL13-PE38 could be achieved at the tumor site for a longer period of time when injected IT without diffusing into the blood circulation. Thus, IT route may avoid repeat cycle avoiding immunity to IL13-PE. Furthermore, it takes about 3 to 4 weeks for humoral immune response to set in and, thus, repeat administration
may be given within 3 weeks for optimal antitumor response. Finally, as IT administration may cause little diffusion to blood circulation antibody titers may be significantly lower in the systemic circulation but not enough to neutralize the activity at the tumor site. These issues should be further tested in the clinical trial.

In conclusion, 40% OSCC tumors strongly positive for IL-13Rα2 may be targeted by IL13-PE38. In addition, as combination therapy with IL13-PE38 and paclitaxel can mediate a synergistic antitumor effect in 2 oral cancer models in mice (subcutaneous and orthotopic tongue xenografts) and approximately 66% of mice receiving combination therapy show complete eradication of tumors, it is possible that this combination will be beneficial in clinical trials. Finally, as retroviral vector-mediated gene delivery of IL-13Rα2 followed by IL13-PE38 administration effectively eliminated established IL-13Rα2 positive and negative OSCC tumors in mice, a combination approach of gene transfer followed by IL13-PE38 therapy will be another option for OSCC therapy. Thus, oral cancers could be a potential target for the combination of IL13-PE38 therapy with either paclitaxel or IL-13Rα2 gene transfer.

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