Targeting IL-13R α_2 for effective treatment of malignant peripheral nerve sheath tumors in mouse models

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OBJECTIVE Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas that harbor a high potential for metastasis and have a devastating prognosis. Combination chemoradiation aids in tumor control and decreases tumor recurrence but causes deleterious side effects and does not extend long-term survival. An effective treatment with limited toxicity and enhanced efficacy is critical for patients suffering from MPNSTs.

METHODS The authors recently identified that interleukin-13 receptor alpha 2 (IL-13R α_2) is overexpressed on MPNSTs and could serve as a precision-based target for delivery of chemotherapeutic agents. In the work reported here, a recombinant fusion molecule consisting of a mutant human IL-13 targeting moiety and a point mutant variant of *Pseudomonas* exotoxin A (IL-13.E13 K-PE4E) was utilized to treat MPNST in vitro in cell culture and in an in vivo murine model.

RESULTS IL-13.E13 K-PE4E had a potent cytotoxic effect on MPNST cells in vitro. Furthermore, intratumoral administration of IL-13.E13 K-PE4E to orthotopically implanted MPNSTs decreased tumor burden 6-fold and 11-fold in late-stage and early-stage MPNST models, respectively. IL-13.E13 K-PE4E treatment also increased survival by 23 days in the early-stage MPNST model.

CONCLUSIONS The current MPNST treatment paradigm consists of 3 prongs: surgery, chemotherapy, and radiation, none of which, either singly or in combination, are curative or extend survival to a clinically meaningful degree. The results presented here provide the possibility of intratumoral therapy with a potent and highly tumor-specific cytotoxin as a fourth treatment prong with the potential to yield improved outcomes in patients with MPNSTs.

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KEYWORDS MPNST; interleukin-13 receptor alpha 2; intratumoral treatment; *Pseudomonas* toxin; murine model; oncology

MALIGNANT peripheral nerve sheath tumors (MPNSTs) constitute a rare and aggressive class of soft tissue sarcoma^{8,41} that can develop after radiation or occur sporadically.³⁴ Almost 50% of MPNSTs are caused by neurofibromatosis type 1 (NF1).⁴⁰ NF1 is an autosomal dominant disorder that occurs in 1 of 3000 live births and is characterized by numerous neurofibromas, cutaneous hyperpigmentation, and intellectual deficits.⁴ The lifetime risk of a person with NF1 for developing an

MPNST ranges between 8% and 13%.⁴⁴ The aggressive nature of MPNSTs is illustrated by the outcome in cases of unresectable MPNST, with a median progression-free survival of less than 2 months and overall survival of less than 5 months.¹⁷ Resective surgery, sometimes requiring complete limb amputation as the only chance at survival, is the standard therapeutic regimen.^{20,37} Tumor control and decreased tumor recurrence can be obtained with combination chemoradiation, but these treatments have not

ABBREVIATIONS ALT = alanine transaminase; AST = aspartate transaminase; BUN = blood urea nitrogen; DMEM = Dulbecco's modified Eagle medium; FBS = fetal bovine serum; H & E = hematoxylin and eosin; hIL-13 = human IL-13; IL-13 = interleukin-13; IL-13R α_2 = IL-13 receptor alpha 2; MPNST = malignant peripheral nerve sheath tumor; NF1 = neurofibromatosis type 1; PBS = phosphate-buffered saline; TCGA = The Cancer Genome Atlas.

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been demonstrated to extend patient survival in the long term.^{35,42} The limited effectiveness of the current therapeutic modalities for MPNST highlights the necessity for effective new treatment modalities for patients suffering from this disease.

Expression of interleukin-13 (IL-13) receptors on MPNSTs has been demonstrated recently.²⁶ There are 2 principal classes of receptor molecules for IL-13. The first of these, a 48-kD protein designated IL-13 receptor alpha 2 (IL-13R α_2), binds weakly to IL-13. Activation of IL-13R α_1 upregulates STAT6, leading to caspase 3–mediated apoptosis.¹³ The second receptor molecule is IL-13R α_2 , a 44-kD protein with 33% homology and 21% sequence identity to IL-13R α_1 ,⁴⁵ but a significantly higher affinity for IL-13. IL-13R α_2 is expressed at substantial levels in glioblastomas, sarcomas (including MPNSTs), and several other tumor types, but it is expressed minimally in nonmalignant tissues except testicular tissue. IL-13R α_2 is a receptor that acts as a decoy, allowing cancer cells to evade death by binding the IL-13 without α_1 activation.¹³ Decreased α_1 activation enables the aggressive nature of MPNSTs by enhancing tumor cell survival and augmenting the already high potential for metastasis and devastating prognosis.^{13,18,19} IL-13R α_2 also acts in an oncogenic fashion by increasing activation of STAT3, ERK, PI3K, and AKT, enhancing cancer invasion and metastasis.^{10,14,29} We have recently discovered that MPNSTs express IL-13R α_2 .²⁶ We have also shown that IL-13–targeted liposomes can be used to improve outcome in a preclinical model for MPNSTs.²⁶ This finding suggests that targeted therapy using an IL-13 variant specific for IL-13R α_2 would be an effective therapeutic modality for this cancer type.

The tumor treatment regimen used for the work reported here involved intratumoral administration of a recombinant fusion protein consisting of an N-terminal targeting moiety (human IL-13 [hIL-13]) with a single point mutation, the C-terminus of which is fused to the N-terminus of a full-length *Pseudomonas* exotoxin A containing 4 point mutations.³³ The point mutations of exotoxin A greatly enhance the affinity for the tumor-specific IL-13R α_2 ³³ and reduce toxicity to nonmalignant cells about 100-fold.^{3,15,22,36,}

When IL-13.E13 K-PE4E binds to an IL-13R α_2 receptor subunit on a malignant target cell, the molecule is internalized¹⁵ and irreversibly disables elongation factor 2, causing the disruption of protein synthesis and eventual cell death.² IL-13.E13 K-PE4E has been shown to be a potent in vitro killer of tumor cell lines cultured from pancreatic tumors,²¹ renal cell carcinoma,³³ squamous cell head and neck tumors,¹⁶ and glioblastomas.²³

A translationally accurate therapeutic model is essential for a clinically predictive evaluation of efficacy both of a therapeutic modality that targets tumor cells expressing IL-13R α_2 and of a treatment regimen that utilizes an intratumoral injection approach. The murine model created for the study described here utilizes mice with orthotopic xenograft of human cultured MPNST cells implanted by intraneural injection into the mouse sciatic nerve.³¹ The MPNST cells were transfected with the constitutively active firefly luciferase gene prior to implantation, in order to enable imaging by bioluminescence to determine the

sizes and locations of the resulting tumor.³¹ Intraneural injections produce a robust tumor in a physiologically relevant microenvironment. We present here the preclinical study of intratumoral IL-13.E13 K-PE4E for the treatment of MPNSTs.

The current standard of care for localized MPNSTs is resection;^{7,11} a retrospective review of 23 cases of MPNST in adults showed resection to provide a significant long-term survival benefit.⁷ Radiotherapy may be useful in local control and may delay the onset of relapse,⁹ but it is accompanied by an elevated risk of radiation-induced sarcomas.⁸ Adjuvant chemotherapy is primarily used for the treatment of diffuse or metastatic disease.⁹ The results of the study described here suggest that therapy with a targeted cytotoxin may have a useful role in the treatment of MPNSTs.

Methods

Cell Culture and Materials

An inoculum of the STS26T human sarcoma cell line, which originated from a patient with sporadic MPNST not associated with NF1 that nevertheless expressed neurofibromin 1,^{5,27} was donated generously by Dr. Daniel Scoles, University of Utah. The cell line was cultured in Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gemini). The cell line was maintained at 37°C in a humidified incubator with 5% CO₂. This cell line was transfected with luciferase as described previously.^{26,31} IL-13.E13 K-PE4E was a generous gift from Targeptics, Inc.

Cytotoxicity of IL-13.E13 K-PE4E In Vitro

STS26T MPNST cells were plated in 96-well plates at 4×10^3 cells per well and cultured for 24 hours. After the 24-hour incubation period, the cells were treated with IL-13.E13 K-PE4E solubilized in 0.1% human serum albumin in phosphate-buffered saline (PBS; Gibco) and diluted to concentrations of 0, 1, 10, 100, 1000, 2000, and 4000 ng/mL. After a 48-hour incubation, an MTS assay was performed according to the manufacturer's protocol (Promega) to determine cytotoxicity through the quantification of viable cells. The measurement was made with quintuplicate well replicates and data were expressed as a percentage of values to untreated cells.

Immunocytochemistry

STS26T cells were plated at 2×10^4 cells per well in a volume of 500 μ L on chamber slides and cultured for 24 hours. The cells were then washed once with PBS for 5 minutes, fixed with 4% paraformaldehyde for 20 minutes, and again washed for 5 minutes thrice with PBS. The slides were then incubated with 10% normal goat serum (Vector Labs) diluted in PBS for 30 minutes at 37°C in a humidified incubator with 5% CO₂. Subsequently the cells were incubated overnight with anti-IL-13R α_2 (1:200 sc-134363) at 4°C. The next day the cells were washed for 5 minutes thrice with PBS. The slides were then incubated with secondary Alexa Fluor 488 conjugated anti-mouse IgG for 60 minutes, then washed thrice with PBS before gel mounting and viewing under a fluorescence microscope (Nikon Eclipse 80i).

FIG. 1. A: Schematic for intratumoral administration of drug. The stars indicate entry sites for injections. Each color represents both poles of a different injection path to be performed on different treatment days. (Example: On day 1 of treatment, the *blue path* was followed and both poles of the tumor along the nerve axis would be treated.) Within each path there are 12 “spots” (6 on each tumor pole). Each *spot* represents a location to which drug was administered. A 7.5- μ L volume of drug was drawn into a 30-gauge Hamilton syringe, which was inserted into the tumor from one end along the path indicated. At each dot along the path, a 2.5- μ L volume of drug was injected; 30 seconds after injection of the drug, the needle was advanced further into the tumor to the next injection site, at which 2.5 μ L of drug was injected. The process was repeated throughout the injection path. With this treatment scheme involving the injection of drug at a rate of 2.5 μ L/30 seconds, no backflow of drug along the path or leakage out of the tumor was observed. **B:** Schematic of the mechanism of action of cytotoxic IL-13.E13 K-PE4E binding to IL-13R α_2 on the tumor. Figure is available in color online only.

Immunohistochemistry Analysis

Tumor tissues were obtained from mice in each group, frozen, sectioned to 10 μ m using a cryostat, and then subjected to hematoxylin and eosin (H & E) staining and immunohistochemistry. The sections were fixed with 4% paraformaldehyde for 20 minutes and washed for 5 minutes thrice with PBS. The sections were incubated with 10% normal goat serum (Vector Labs) diluted in PBS for 30 minutes at 37°C in an incubator. The slides were then incubated overnight at 4°C with Ki-67 antibody (1:500 ab15580) and cleaved caspase 3 (1:500 9661S), and then washed for 5 minutes with PBS. They were then incubated with secondary Alexa Fluor 488 conjugated anti-rabbit IgG and DAPI for 60 minutes at room temperature and washed for 5 minutes thrice with PBS before gel mount-

ing and viewing under a fluorescence microscope (Nikon Eclipse 80i).

Orthotopic MPNST Tumor Model

The STS26T cell line was cultured until approximately 90% confluency in complete DMEM supplemented with 10% FBS. Cells were washed once with PBS and detached with 1 mL of 1 $\times$ trypsin-EDTA (Gibco). The cells were resuspended in DMEM with 10% FBS (Gemini) and centrifuged at 2400g (IEC Centra CL2). The supernatant was aspirated with a pipette and the cells were resuspended into 50,000 cells/3 μ L of media. This cell resuspension was then aspirated into a 30-gauge Hamilton syringe and used for injection into the mouse sciatic nerve.³¹ We utilized female NIH-III mice from Charles River (age 5–8

FIG. 2. IL-13R α_2 analysis from the TCGA database showing that MPNSTs, which are in the “SARC” category of sarcomas, are in the top 10 for the highest IL-13R α_2 expression of all tumor types. The y-axis denotes log2 expression of IL-13R α_2 in TCGA RNA-Seq datasets. Figure is available in color online only.

weeks, average weight 24 g). These mice have the nude gene and the x-linked immune defect (*xid*) and beige (*bg*) mutations. Two experimental tumor groups, early-stage and middle-late-stage tumor development, were established to represent clinical scenarios. For the early-stage MPNST study, tumors were allowed to grow for 1 week, all mice were then normalized based on bioluminescence counts and randomly distributed into control ($n = 6$) and treatment ($n = 6$) groups, and then treatment began. For the middle-late-stage study, mice were imaged every 2 days with in vivo fluorescence imaging (IVIS 50 imaging system, PerkinElmer) until tumors became the size of 10^5 based on luminescence counts measured with LivingImage software, which took an average of 3.5 weeks. Once the tumors attained this threshold of bioluminescence counts, the mice were ranked in order of bioluminescent signal intensity and then randomly distributed and normalized into 2 homogeneous groups—a control group ($n = 8$) and a treatment group ($n = 6$). The normalized groups then began treatment.

Intratumoral Treatments

The control group received intratumoral injections of 0.1% human serum albumin in PBS equal to the volume administered in the treatment group and at the same intervals. The treatment group received intratumoral IL-13.E13

K-PE4E. For the middle-late-stage MPNST study, intratumoral IL-13.E13 K-PE4E was administered percutaneously without surgery, as shown in Fig. 1A, with a 30-gauge Hamilton syringe.

Figure 1B shows a schematic of the cytotoxic mechanism of action of IL-13.E13 K-PE4E binding to the tumor. After the hIL-13.E13 K-PE4E targeting moiety of the cytotoxin binds to an IL-13R α_2 receptor subunit on the surface of the tumor cell, the molecule is internalized by a process mediated by the intact translocase domain II of the *Pseudomonas* exotoxin A sequence.^{15,28,32,38} Inside the target cell, the catalytic domain III of the exotoxin A is cleaved by the intracellular protease furin.^{12,43} This cleavage releases the active enzyme ADP-ribosyl transferase; this enzyme ribosylates and thus irreversibly activates eukaryotic elongation factor 2.^{2,30} The inactivation of this protein halts protein synthesis, ultimately causing cell death.

For the induction phase of treatment, 1 μ g of IL-13.E13 K-PE4E in 30 μ L of PBS was administered a total of 3 times 3 days apart (Monday/Thursday/Sunday); after a 1-week rest period, the mice were then treated with maintenance doses of 1 μ g of IL-13.E13 K-PE4E in 30 μ L twice per week. For the early-stage MPNST study, 1 week after orthotopic tumor cell implantation, the orthotopic tumors were surgically exposed with the aid of a surgical micro-

FIG. 3. Follow-up IL-13R α_2 analysis of the SARC category from the TCGA database (Fig. 2) showing that MPNSTs are the top IL-13R α_2 expressers in this category. The y-axis denotes log2 expression of IL-13R α_2 in TCGA RNASeq datasets. mfh = malignant fibrous histiocytoma. Figure is available in color online only.

scope (Zeiss) to provide visualization of the normal nerve and the tumor. Once the tumor was exposed, a 3- μ g dose of IL-13.E13 K-PE4E in 3 μ L was intratumorally administered with a 30-gauge Hamilton syringe using the surgical microscope to provide visual confirmation that treatment in fact did go into the tumor. Thereafter, 3- μ g doses of IL-13.E13 K-PE4E were given 10 days and 20 days after the first treatment in the same fashion, for a total of 3 treatments.

In Vivo Tumor Progression, Survival, and Toxicity Analysis

The mice were anesthetized with isoflurane; an injection of luciferin-D (Caliper LS, 100 μ L, 28.57 mg/mL) was administered subcutaneously for bioluminescent detection of tumors. Luciferin was allowed to circulate for 5 minutes before IVIS imaging was performed. Tumors were exposed for 1 minute and bioluminescence counts were recorded. If a measurable bioluminescence count was detected, the mouse was deemed to have a tumor. If bioluminescence was not observed, the mouse was reimaged immediately with a 1-minute exposure. If after multiple

repeat images no bioluminescence counts were observed, the mouse was considered negative for tumor. Imaging was performed every 2 days for the middle-late-stage study, and twice a week for the early-stage study. Tumor burden was determined through bioluminescence counts. Survival was assessed based upon the number of days after tumor induction at which death occurred—either because the animals died on their own or because the recommended veterinary euthanasia endpoint was reached. This experimental endpoint was defined by the veterinarians as a tumor size large enough to preclude the mouse from walking, eating, and drinking. At the veterinarian-recommended euthanasia endpoint, cardiac puncture was performed and blood was collected. The blood was further analyzed for bioindicators of toxicity, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate transaminase (AST), and alanine transaminase (ALT) by the Penn State Core Facility. The blood was taken to the core facility within 1 hour of blood collection and the Roche Cobas Mira Plus was used to analyze serum samples. This instrument utilizes photometry to measure colorimetric changes in the samples.

FIG. 4. IL-13R α_2 expression and in vitro cytotoxicity following targeting. **A:** MPNST cells with IL-13R α_2 antibody incubation. **B:** MPNST cells incubated without primary IL-13R α_2 antibody, but with secondary antibody to control for IL-13R α_2 antibody specificity. **C:** In vitro cytotoxicity analysis showing that increasing IL-13.E13 K-PE4E concentration causes enhanced MPNST cell death. Bar (A and B) = 100 μ m. Figure is available in color online only.

TCGA Database Analysis

The Cancer Genome Atlas (TCGA) is a joint effort of the National Cancer Institute and the National Human Genome Research Institute to establish a central database of cancer-related genomic data. The TCGA encompasses 33 types of cancer, with data from 11,000 patients. We utilized this broad dataset to assess the log₂ expression of IL-13R α_2 in TCGA RNASeq datasets. Specifically, we determined the 10 cancer types with highest IL-13R α_2 expression, and we further analyzed for highest IL-13R α_2 expression in the sarcoma category.

Statistical Analysis

All of the data generated in the experiments were subjected to statistical analysis using GraphPad Prism 4.03 software package. At least 3 replicates were performed unless otherwise noted, and groups were compared using unpaired t-tests. A *p* value < 0.05 was deemed significant.

Results

IL-13R α_2 Expression in the TCGA Database

The scatter diagram presented in Fig. 2 is a plot of levels of expression of IL-13R α_2 for individual patients across 33 tumor types, using data from TCGA. The y-axis denotes log₂ expression of IL-13R α_2 in TCGA RNASeq datasets from the cancer types. It can be seen that as a group, sarcomas (“SARC”), of which MPNSTs are a sub-

set, are the 10th highest expressers of IL-13R α_2 (red box, Fig. 2) among the 33 cancer subtypes represented in the TCGA database.

The scatter diagram in Fig. 3 is a follow-up plot showing levels of expression of IL-13R α_2 within the SARC category alone from the TCGA data in Fig. 2. It can be seen that in this SARC group, MPNSTs are the highest expressers of IL-13R α_2 within the SARC category (red box, Fig. 3).

IL-13R α_2 Expression in MPNST Cells and IL-13.E13 K-PE4E Cytotoxicity In Vitro

The presence of IL-13R α_2 can be demonstrated on the MPNST cells with immunocytochemistry (Fig. 4A). Cells incubated in the absence of primary antibody exhibited no fluorescence; these samples served as a negative control for antibody (Fig. 4B). The bar graph in Fig. 4C demonstrates that the killing of STS26T MPNST cells in culture by IL-13.E13 K-PE4E is dose-dependent.

In Vivo Analysis of IL-13.E13 K-PE4E

We assessed the effectiveness of intratumoral IL-13.E13 K-PE4E in treating middle-late-stage MPNSTs as well as early-stage MPNSTs (Fig. 5). IL-13.E13 K-PE4E significantly decreased tumor progression 6-fold in the middle-late-stage model in comparison to controls (Fig. 5A, *p* < 0.05); however, overall survival was not significantly increased (Fig. 5B). IL-13.E13 K-PE4E significantly decreased tumor progression 11-fold in the early-stage model (Fig. 5C, ***p* < 0.01). The IL-13.E13 K-PE4E-treated group in the early-stage tumors also had a significantly increased survival by 23 days (Fig. 5D, *p* = 0.0015). The median survival was 51 days for the control group and 74 days for the IL-13.E13 K-PE4E-treated group.

Mice were sacrificed at the endpoint determined by the veterinarians, blood was drawn immediately, and samples were taken to the core facility for toxicity analysis within 1 hour of being drawn. Blood chemistries were assessed for nephrotoxicity (Fig. 6A and B) and hepatotoxicity (Fig. 6C–E). The serum levels of blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate transaminase (AST), and alanine aminotransferase (ALT) did not differ significantly between the control and IL-13.E13 K-PE4E-treated groups (Fig. 6A–E). The IL-13.E13 K-PE4E-treated mice did have a 1- to 2-g decrease in overall weight compared to the saline-injected controls (Fig. 6F). The lack of significant increases in the enzymes tested suggests that treatment with IL-13.E13 K-PE4E did not have detectable toxic effects on renal or hepatic function.

H & E and Immunohistochemistry on Tumor Tissue

Tumors treated with the IL-13.E13 K-PE4E or control were excised, and H & E and immunohistochemical analysis was performed to evaluate cellular architecture and necrosis in the MPNSTs. Extensive areas of vacuolization are denoted in the tumors treated with IL-13.E13 K-PE4E compared to control (Fig. 7A). Ki-67 immunostaining was robust in the nontreated MPNSTs but rarely detected in the IL-13.E13 K-PE4E-treated tumors (Fig. 7B). Cleaved caspase 3 was sparsely detected in the nontreated tumors

FIG. 5. In vivo tumor progression and survival. **A:** Middle-late-stage MPNST mice treated with intratumoral IL-13.E13 K-PE4E exhibited significant decreases in tumor burden in comparison to controls. **B:** Duration of survival did not differ significantly between the treated and control late-stage tumor groups. It should be noted that although some of the animals died of disease progression, most were euthanized because they had reached the predetermined veterinary euthanasia endpoint. See *Methods* for explanation of endpoint. **C:** Early-stage tumor mice treated with IL-13.E13 K-PE4E exhibited significant decreases in tumor burden in comparison to controls. **D:** Duration of survival was significantly increased in the early-stage treatment mice. Median survival times for the control and IL-13.E13 K-PE4E treated groups were 51 and 74 days, respectively (* $p < 0.05$, ** $p < 0.01$). Figure is available in color online only.

but was robust in the IL-13.E13 K-PE4E-treated MPNSTs (Fig. 7C).

Discussion

The data presented from the TCGA database indicate that sarcomas—the tumor class that includes MPNSTs—are among the higher tumor expressers of the tumor-selective IL-13 α_2 receptor subunit. Furthermore, of tumors in the sarcoma category, MPNSTs have the highest expression of IL-13 α_2 . The results of the studies performed show that IL-13.E13 K-PE4E effectively kills MPNST cells in vitro and in vivo and thus should be considered as a viable drug for the clinical treatment of MPNSTs. Moreover, the results demonstrated the effectiveness of our intratumoral injection technique for achieving localized drug delivery; the results of tests for serum indicators of kidney and liver function suggest that this technique caused minimal systemic exposure to the drug.

The elevated expression of IL-13 α_2 by MPNSTs provides an opportunity for utilizing IL-13.E13 K-PE4E to selectively target MPNST cells and cause direct tumor cell death. This approach in our study can be translated into

the clinic. Intratumoral delivery of drug can be performed under local anesthesia with ultrasound guidance and thus may provide a minimally invasive and effective novel therapeutic modality for MPNSTs. The minimally invasive nature of intratumoral delivery may be critical especially for patients who are not surgical candidates because of preexisting conditions or patients who have MPNSTs in critical nerves that are not amenable to resection. The mutant form of IL-13 used in this therapeutic agent is more selective for its receptor than the wild-type IL-13 that was tested clinically in glioblastomas.²⁴ The specific targeting of IL-13 α_2 with mutant peptides of IL-13 may also lead to an improved side effect profile due to minimal IL-13 α_2 expression in normal tissues other than testicular tissue.²⁵

A challenge for intratumoral chemotherapy is the high likelihood of backflow of the drug through the needle track. Previous intratumoral methodologies inject drug to the center of the tumor and rely on passive diffusion for distribution of the drug throughout the tumor tissue; this objective is difficult to achieve in most solid tumors. The method of intratumoral injection described here results in minimal backflow and achieves delivery of drug to multiple loca-

FIG. 6. Blood toxicity analysis. Tests for kidney toxicity, with BUN and creatinine, as well as liver function, with alkaline phosphatase, AST, and ALT, were performed. **A and B:** BUN and creatinine showed no significant increase in toxicity due to IL-13.E13 K-PE4E treatment. **C–E:** Alkaline phosphatase, AST, and ALT showed no significant increase in toxicity due to IL-13.E13 K-PE4E treatment. **F:** The IL-13.E13 K-PE4E-treated mice had a 1- to 2-g decrease in overall weight compared to the saline controls. Figure is available in color online only.

tions within the tumor by using multiple treatment trajectories. We chose an injection technique involving gradual penetration of the tumor tissue, with partial injections at intervals during penetration, rather than immediately penetrating to the tumor center, because use of the latter method would result in the dead space volume of the needle track becoming a reservoir of IL-13.E13 K-PE4E. By only injecting at a rate of 2.5 μ L/30 seconds, no backflow of drug out of the tumor was observed. By avoiding backflow, and thus keeping the drug in the tumor, we appear to have overcome the historically critical problem of backflow with intratumoral injections, resulting in more effective treatment of the tumor and decreasing the side effect profile.

Although we attempted to inject the chemotherapeutic agent throughout the entire tumor, it was difficult to inject drug at the outer tumor borders. This inability to treat external regions may result in persistent vascularization and cell proliferation at the periphery of the tumor and is the likely reason for the differences observed in tumor regression versus overall survival in the late-stage treatment group. Our veterinarians determine when the mice have reached their endpoint, and this decision is solely based on visual inspection of tumor size. Thus animals reached the endpoint of the study even though there were fewer bioluminescent counts, meaning less viable tumor, as shown by Fig. 5B. As a future direction, we will explore the use of radiotherapy in combination with intratumoral IL-13.E13 K-PE4E because radiation treatment may be able to target the periphery of the tumor, an approach that would likely be used clinically.

Because we expect this compound and surgical approach

to be translated to the clinic, we address the question of the suitability of preclinical models. Human and murine IL-13 cytokine-receptor relationships have been extensively studied. Previous work has shown a 59% primary sequence identity between murine and human IL-13 α_2 subunits⁶ and found 76% structural homology.¹ Most importantly, structure is highly conserved between the 2 species, and IL-13 binding specificity is similarly high for both. Human and murine IL-13 receptors both have short cytoplasmic domains, are lacking the same signaling motifs, and share 95% primary sequence identity.^{1,6} Moreover, sequence homology between α_1 and α_2 receptor subunits is low ($\approx$ 25%) in both species;³⁹ as would be expected, most of this homology occurs in the extracellular binding domain. Given the high degree of structural and sequence homology between the IL-13 α_2 subunits in mice and humans, it is reasonable to expect that efficacy and toxicity data obtained in these and subsequent *in vivo* investigations will be relevant to the clinical experience encountered in the treatment of MPNSTs in human patients.

Conclusions

IL-13 α_2 is a tumor-associated receptor subunit for IL-13 that is highly expressed on MPNSTs. Recent studies in our laboratory have demonstrated doxorubicin-loaded liposomes conjugated to wild-type IL-13 to be more effective in treating MPNSTs and gliomas than doxorubicin alone,²⁶ and the present study further suggests the potential effectiveness of drugs targeting IL-13 α_2 for MPNST treatment. The use in this study of our previ-

FIG. 7. Tumor tissue analysis. **A:** Aberrant cellular architecture and robust necrosis in the MPNSTs treated with intratumoral IL-13.E13 K-PE4E in comparison to intratumoral saline control. Extensive areas of vacuolization are present (denoted with arrows). **B:** Ki-67 immunostaining was robust in the nontreated MPNSTs but rarely detected in the IL-13.E13 K-PE4E–treated tumors, suggesting a decrease in cellular proliferation. **C:** Cleaved caspase 3 was sparsely detected in the nontreated tumors but was robust in the IL-13.E13 K-PE4E–treated MPNSTs, suggesting an increase in tumor cell death. Bar = 100 µm. Figure is available in color online only.

ously described orthotopic murine model³¹ to demonstrate the effectiveness of IL-13.E13 K-PE4E as a treatment for MPNST is a novel approach. The results of our in vivo investigation suggest that treatment with IL-13.E13 K-PE4E leads to a decrease in tumor progression and increase in survival. The current standard of care for MPNST consists of 3 therapeutic modalities: surgery as a first line of therapy, followed by adjunctive radiotherapy, and then chemotherapy for diffuse or metastatic tumors, all of which have been demonstrated to be unsatisfactory. The results presented here present the possibility that the use of localized intratumoral administration of IL-13.E13 K-PE4E may be an effective treatment for malignant MPNST cells and might be able to meaningfully advance the standard of care for patient with MPNSTs.

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Disclosures

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Conception and design: Mrowczynski, Payne, Abramson, Wolfe, Harbaugh, Rizk, Connor. Acquisition of data: Mrowczynski, Payne, Bourcier, Mau, Slagle-Webb, Shenoy, Madhankumar. Analysis and interpretation of data: Mrowczynski, Payne, Abramson, Wolfe, Rizk, Connor. Drafting the article: Mrowczynski, Rizk. Critically revising the article: Abramson, Wolfe, Harbaugh, Rizk, Connor. Reviewed submitted version of manuscript: Mrowczynski, Abramson, Wolfe, Harbaugh, Rizk, Connor. Approved the final version of the manuscript on behalf of all authors: Mrowczynski. Statistical analysis: Mrowczynski, Payne. Study supervision: Abramson, Wolfe, Harbaugh, Rizk, Connor.

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