Original Study

Anti-Mesothelin Recombinant Immunotoxin Therapy for Colorectal Cancer

Adam Cerise,[1] Tapan K. Bera,[1] Xiufen Liu,[1] Junxia Wei,[1] Ira Pastan[1]

Abstract

Mesothelin is expressed at high levels on the surface of colorectal carcinoma cells, mesothelioma, and pancreatic, ovarian, and gastric cancer. Immunotoxins targeting Mesothelin can kill colorectal cancer cell lines in vitro and can inhibit growth and cause regressions in mice. Combination therapy with actinomycin D resulted in > 90% tumor volume reduction with 50% complete regressions.

Background: Mesothelin (MSLN) is a cell surface glycoprotein expressed at a high level on many malignancies, including pancreatic adenocarcinoma, serous ovarian cancer, and epithelioid mesothelioma. MSLN-targeted recombinant immunotoxins (RITs) consist of an anti-MSLN Fv fused to the catalytic domain of *Pseudomonas* exotoxin A. Recent data has also shown that MSLN is expressed at clinically relevant levels on the surface of colorectal cancer (CRC). In this study, CRC cell lines were tested for MSLN expression and susceptibility to MSLN-targeted RITs. **Materials and Methods:** CRC cell lines were tested for membranous MSLN expression via flow cytometry. Cell lines expressing MSLN were tested by WST-8 cell viability assay for sensitivity to various RITs and chemotherapeutic agents. CRC cell line SW-48 was tested in a mouse model for response to RIT as a single agent or in combination with actinomycin D and oxaliplatin. **Results:** CRC cell lines were susceptible to anti-MSLN RITs at half maximal inhibitory concentration levels comparable with those previously described in pancreatic cancer cell lines. In a nude mouse model, MSLN-targeted RIT treatment of SW48 CRC tumors resulted in a significant decrease in tumor volume. Although combination therapy with standard of care chemotherapeutic oxaliplatin did not improve tumor regressions, combination therapy with actinomycin D resulted in > 90% tumor volume reduction with 50% complete regressions. **Conclusions:** These data support the development of anti-MSLN RITs as well as other MSLN-targeted therapies for CRC.

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Introduction

A recombinant immunotoxin (RIT) is a chimeric protein consisting of the Fv or Fab portion of an antibody fused to a truncated bacterial toxin. In our laboratory, we use *Pseudomonas* exotoxin A (PE) as the toxin. To construct RITs, we replace domain I, the binding domain of PE, with an Fv or Fab portion of an antibody, which binds to a cancer cell-specific surface antigen. After binding to the cell, the toxin is internalized, arrests protein synthesis, and kills the cell. Because protein synthesis is necessary for cell survival, RIT therapy can kill both dividing and quiescent cells.[1]

An RIT containing an anti-CD22 Fv fragment, clinically identified as Moxetumomab pasudotox, has shown considerable efficacy in phase I and phase III studies in drug-refractory hairy cell leukemia, including a 64% complete response rate and an 88% overall response rate.[2,3] Moxetumomab pasudotox is now United States Food and Drug Administration-approved for the therapy of drug-resistant hairy cell leukemia. However, targeting solid organ epithelial cancers is more challenging than targeting leukemias. One attractive target is mesothelin (MSLN), a 71 kDa glycoprotein that is cleaved by furin into a 40 kDa membrane-bound form, expressed on the cell membrane of mesothelial cells lining the peritoneum, pleura, and pericardium, and a secreted 31 kDa megakaryocyte potentiating factor whose function has yet to be clearly elucidated. MSLN was originally found to be highly expressed on the cell

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membrane of ovarian cancer OVCAR-3 cells.[4] Since then, MSLN has been studied extensively and found to be highly expressed on many malignancies, including pancreatic adenocarcinoma and mesothelioma.[5-8] An RIT targeting MSLN named SS1P was constructed by fusing the Fv portion of an anti-MSLN[9] antibody to a 38 kDa fragment of PE.[9] SS1P displayed a favorable safety profile in phase I trials and was investigated in a cohort of mesothelioma patients.[10] However, efficacy was limited by the development of neutralizing antibodies in 90% of treated patients.[10] In a subsequent study in which immunogenicity was mitigated with a lympho-depletive regimen prior to administration of RIT, major regressions lasting more than 20 months were observed in 3 of 10 patients with chemotherapy-resistant malignant mesothelioma.[11]

Another approach to prevent the production of anti-drug antibodies has been to remove or modify B cell epitopes in the RIT. In collaboration with Roche pharmaceuticals, RG7787, or LMB-100, was developed. In LMB-100, the mouse Fv is replaced by a humanized Fab,[12] most of domain II is removed, and mutations introduced into domain III to inactivate B cell epitopes. LMB-100 has been found to be very cytotoxic to many human cancer cell lines expressing MSLN and causes regressions of several types of MSLN-expressing cancers.[12] LMB-100 is currently in phase I/II trials at the National Cancer Institute (NCI) for mesothelioma and a variety of MSLN-expressing gastrointestinal malignancies. We have also developed an anti-MSLN RIT that contains an albumin binding domain that greatly increases half-life in the circulation.[13] In the current study, we have determined if these 2 RITs are effective in killing colon cancer cells in culture and reducing the growth of colon cancer xenograft in mice.

Materials and Methods

Cell Culture and Reagents

HTB39, KLM-1, and SW48 cell lines were purchased from ATCC, and have been previously described.[14] Identity of all cell lines was confirmed by STR testing. STR refers to short tandem repeat DNA profiling that identifies human cell lines derived from individual tissue, ensuring purity of cultures and lack of cross-contamination.

KLM-1 cells were cultured in RPMI 1640 medium (Gibco, Thermo Scientific) supplemented with L-Glutamine (2 mmol/L), penicillin (100 U), streptomycin (100 µg), and 10% fetal bovine serum (Hyclone, Thermo Scientific). HTB39 and SW48 cell lines were grown at 37°C with 5% CO_2 in D-MEM medium (Gibco Life Technologies) supplemented with 100 U penicillin, 100 µg streptomycin (Gibco Life Technologies), and 10% fetal bovine serum (Thermo Scientific). LMB-100 was manufactured by Roche and provided for these studies through a Collaborative Research and Development Agreement. LMB-164 and LMB-12 were prepared in our laboratory as previously described.[13] Actinomycin D was obtained through Sigma Pharmaceuticals. Oxaliplatin was obtained through the National Institutes of Health (NIH) DVR Animal Pharmacy and was manufactured by TEVA Pharmaceuticals.

MSLN Surface Expression Assay

CRC cell lines were assayed for membrane MSLN expression via flow cytometry. 20,000 cells per sample were analyzed on a FACSCalibur instrument (BD Bioscience) running CellQuest software (BD Bioscience). Data processed using FlowJo software (Tree Star Inc). Cells were plated and grown for 48 hours in culture, then harvested with trypsin. Live cells were washed and fixed with FACS buffer, then incubated with mouse anti-MSLN antibody (BioXcell) for 30 minutes. Cells were subsequently washed again and incubated for 30 minutes in darkness with PE conjugated antibody Fragment Goat anti-mouse IgG (Jackson Immuno Research).

In Vitro Quantitative Cell Viability Assay

The viability of cells treated with RIT and chemotherapy was measured using Cell Counting Kit WST-8 Assay (Dojindo Molecular Technologies, Inc). All cell lines were plated at 5000 cells per well in 96-well plates and incubated at 37°C for 24 hours prior to treatment. RIT and chemotherapy treatments were diluted in complete medium and added to wells in indicated concentrations. Cells were incubated at 37°C for 72 hours, then treated with WST-8 assay reagent per manufacturer's instructions. Plates were incubated at 37°C for 4 hours, and absorbance at 450 nm was measured. Values normalized between 0% viability for Staurosporine treatment (Sigma Pharmaceuticals), which produces total cell killing, and medium alone for 100% cell viability. Data were then plotted in GraphPad Prism 6 for fit curve and interpolated half maximal inhibitory concentration (IC_{50}) value determination.

Mouse Xenograft Tumor Model

All animal experiments were performed in accordance with NIH guidelines and approved by the NCI Animal Care and Use Committee. SW48 cells (4×10^6) supplemented with Matrigel (4.0 mg/mL) were injected subcutaneously into the right flank of 6- to 8-week-old female, athymic nude mice. Tumors were allowed to grow to an average of 100 mm^3 prior to treatment initiation. Actinomycin D (0.3 mg/kg) was diluted in 0.2% human serum albumin (HSA) in Dulbecco's phosphate-buffered saline (D-PBS) and injected intra-peritoneally on days indicated. All RITs were diluted in D-PBS and administered intravenously (IV) through the tail vein on days indicated. Oxaliplatin (4.2 mg/kg) was diluted in 5% dextrose solution and administered IV through the tail vein on days indicated. Tumor size was measured in 2 dimensions by digital calipers at least twice weekly, and volume was calculated using the formula: $0.4 \times \text{width}^2 \times \text{length}$. Animals were sacrificed before tumor volume reached 1000 mm^3 or tumors developed necrotic ulcerations. Data were recorded, and tumor growth curves were plotted in Microsoft Excel and GraphPad Prism 6 for interpolation and statistical analysis.

Statistics

Excel and GraphPad Prism 6 were used for all statistical calculations and curve fitting. Errors are reported as standard deviations. The 2-tailed Student t test was used for statistical comparisons. Significance was established at $\alpha \leq 0.01$.

Results

MSLN Expression in CRC Cell Lines

Although MSLN expression was previously analyzed in tissue micro-array data of CRC and considered relatively low, a recent analysis of the frequency in which MSLN protein can be detected in

CRC samples is much higher, showing that almost two-thirds of CRC surgical specimens are positive.[15,16] Further, the analysis of mRNA expression of MSLN in various carcinomas from the Cancer Cell Line Encyclopedia (CCLE) database (http://www.broadinstitute.org/ccle) shows expression levels on par with those of ovarian cancer and gastric adenocarcinoma (Figure 1A). To identify CRC cell lines, which could be used in a model to test MSLN-targeted RITs, we examined surface MSLN expression in established CRC cell lines using FACS analysis with an antibody to MSLN.[12] Figure 1B shows histograms of surface MSLN expression of 2 CRC cell lines, HTB39 and SW48. Both cell lines were uniformly positive, indicating they should be susceptible to MSLN-targeted RITs.

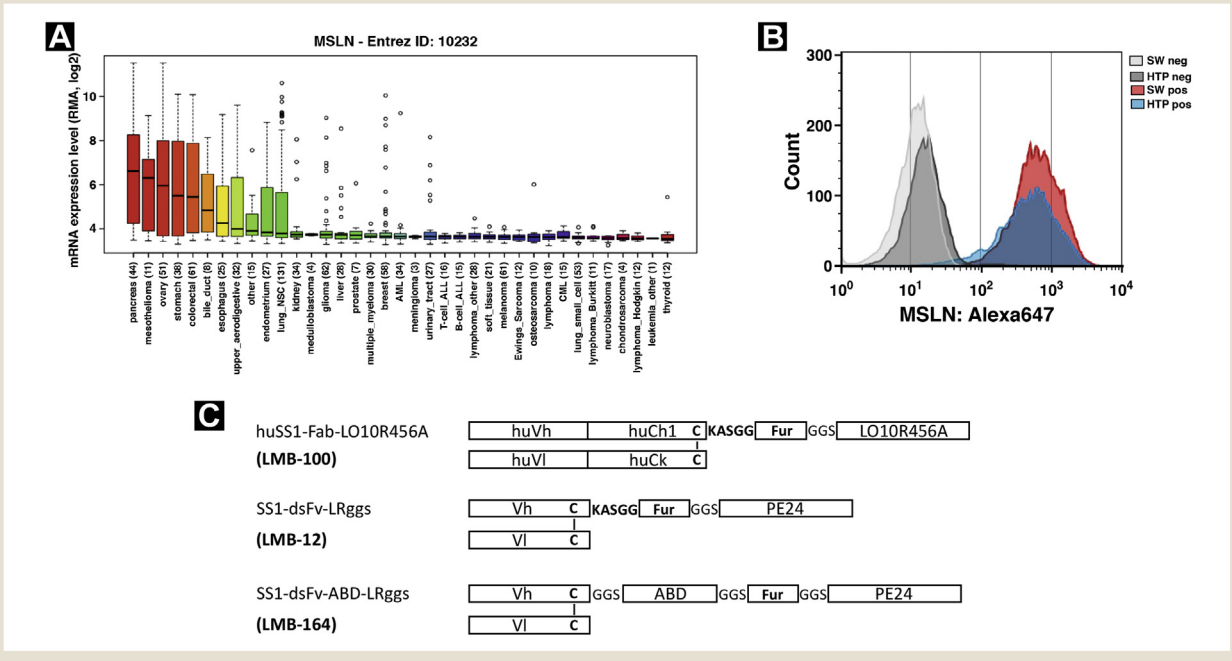
Sensitivity of CRC Cell Lines to MSLN-targeted RITs

We tested HTB39 and SW48 cell lines for RIT sensitivity using various anti-MSLN RITs: LMB-12, LMB-100, and LMB-164 (Figure 1C). LMB-12 consists of an anti-MSLN dsFv fragment fused to domain III of PE. LMB-100 consists of humanized Fab and mutations in domain III that silence many of B and some T cell epitopes to reduce immunogenicity.[17] LMB-164 is based on LMB-12 but has an albumin binding domain (ABD) that increases half-life.[13] Sensitivity to these RITs was compared with that of KLM-1 pancreatic adenocarcinoma cells. The data in Figure 2 and Table 1 show that SW48 and HTB39 cells are sensitive to all 3 RITs. The IC_{50} on SW48 cells for LMB-12 is 0.37 ng/mL, for LMB-100 is 0.78 ng/mL, and for LMB-164 is 0.55 ng/mL. HTB39 cells are somewhat less sensitive than SW48 cells but are also killed by all 3 agents. The IC_{50} values are 2.7 ng/mL for LMB-12 and LMB-100; 5.7 ng/mL for LMB-164. No significant difference in RIT sensitivity was noted between SW48 cells and that of KLM-1 for any of the RITs. Immunotoxin LMB-11(HA22-Fab-LO10R456A) targeting CD22 antigen, which is not expressed in colon cancer or pancreatic cancer cell lines, was used as negative control. As expected, LMB-11 has no killing activity on these colon cancer cell lines at 1000 ng/mL concentration (Table 1).

LMB-100 and LMB-164 Efficacy in CRC Mouse Xenograft Model

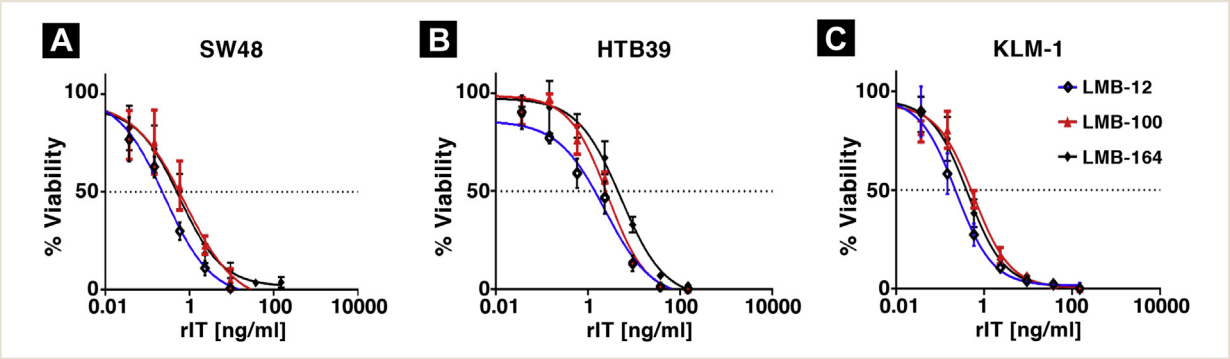
Given the favorable sensitivity of SW48 cells to anti-MSLN RITs, the in vivo efficacy of LMB-100 and LMB-164 in an SW48 mouse xenograft model was evaluated. Athymic nude mice

Figure 1 **MSLN Expression and MSLN-targeted RITs. A, Expression of MSLN mRNA as Detected in Various Solid Organ Malignancies. Data Were Analyzed From Cancer Cell Line Encyclopedia (CCLE), Representing Gene Expression of 1036 Samples From 36 Tumor Types. B, Histogram of SW48 and HTB39 Colorectal Cancer Cell Lines Tested for Membranous MSLN Expression by Fluorescence-activated Cell Sorting Analysis. Live Cells Incubated With anti-MSLN Primary Antibodies Followed by Incubation With Appropriate *Pseudomonas* Exotoxin a (PE)-conjugated Antibody Detected as FL4-H. A Minimum of 20,000 Cells Were Used for MSLN Detection. C, Schematics of Different anti-MSLN RITs Used in this Manuscript. LMB100 Comprises Humanized SS1 Fab Linked to Furin Cleavage Site (Fur) Followed by Mutated Version of PE Toxin, LO10R456A. LMB-12 Comprises SS1disulfied-stabilized Fv Linked to Furin Cleavage Site (Fur) Followed by Mutated Version of PE Toxin, PE24. LMB-164 Comprises SS1disulfied-stabilized Fv Linked to an ABD Followed by Furin Cleavage Site (Fur) and a Mutated Version of PE Toxin, PE24**



Abbreviations: ABD = albumin binding domain; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CML = chronic myeloid leukemia; MSLN = mesothelin; NSC = non–small-cell; RIT = recombinant immunotoxin therapy.

Figure 2 Activity of anti-MSLN Immunotoxin on Colon Cancer Cell Lines. A, KLM-1 Pancreatic Adenocarcinoma Cells Were Treated With Indicated Concentrations of LMB-12, LMB-100, or LMB-164 anti-MSLN Targeted RITs for 72 Hours Before WST-8 Assay of Cell Viability. Vehicle Treated Cells Were Normalized to 1, and Staurosporine Treated Cells Were Normalized to 0. Each Data Point Measures 6 Treated Wells. Identical Assay for SW48 CRC Cell Line (B) and HTB39 CRC Cell Line (C)



Abbreviations: CRC = colorectal cancer; MSLN = mesothelin; RIT = recombinant immunotoxin therapy.

were inoculated in the right flank with SW48 cells on Day 0, and tumors were allowed to grow to an average volume of 100 mm^3 before initiation of treatment on Day 7. LMB-100 was administered IV at 2.5 mg/kg dose every other day for 6 days for 2 cycles with 3 days in between cycles. LMB-164 has an ABD and longer half-life (194 minutes) in mouse serum. LMB-164 was administered IV at 0.3 mg/kg dose every day for 3 days for 2 cycles with 1 day between cycles. These doses were determined to be safe in prior experiments.[13] The data in Figure 3 shows that both LMB-100 and LMB-164 produced tumor regressions. All RIT-treated tumors had regressed by 50% on Day 12. Vehicle (PBS)-treated tumors grew exponentially throughout this time. By the second dose of LMB-100 and the third dose of LMB-164, a significant difference in tumor volume between the vehicle-treated controls and RIT-treated tumors developed. This difference persisted throughout the remainder of the experiment, reaching a maximum at Day 11. In addition, there was a significant delay of 13 days for the tumor volume to reach 300 mm^3 in animals treated with RIT versus vehicle.

Combination Therapy With Actinomycin D

Previously, we have demonstrated that co-administration of actinomycin D with RIT upregulates apoptotic proteins of both the extrinsic and intrinsic pathways and shows significant synergy in vivo in treatment of pancreatic adenocarcinoma.[18] Although actinomycin D has not recently been used in the treatment of colorectal adenocarcinoma, it remains a mainstay of therapy for childhood sarcomas, with a toxicity profile that does not overlap with RIT therapy. Therefore, we tested LMB-100 in combination with actinomycin D using SW48 tumors. Figures 4 and 5 show that combination treatment resulted in a 95% reduction in average tumor volume by Day 19 in comparison with a 40% reduction in tumor size for RIT monotherapy. Actinomycin D alone caused a growth delay shortly after initial dosing, but the tumors did not regress. In addition, combination therapy resulted in complete regressions in 13 of 22 treated mice. Figure 5 shows a spider plot of the tumor volume of an individual mouse from a representative anti-tumor experiment with 4 of 8 complete responses in the combination treatment group. There was no significant weight loss in animals treated with LMB-100 in combination with actinomycin D, although actinomycin D alone caused some weight loss after the second dose (see Supplemental Figure 1 in the online version).

Combination Therapy With Oxaliplatin

Previous data has shown synergy of chemotherapeutic agents with RIT therapy in in vivo models.[19,20] Furthermore, combination screening of various chemotherapeutics noted a positive trend in combination of cisplatin and anti-MSLN targeted RITs.[18] Given the widespread use of oxaliplatin platinum in CRC therapy, we investigated the potential of combing it with anti-MSLN RITs. We first analyzed combination efficacy using live/dead staining flow cytometry and noted a trend toward increasing numbers of dead and apoptotic cells with higher concentrations of oxaliplatin and LMB-100 (See Supplemental Figure 2 in the online version). We then tested the efficacy of oxaliplatin combination therapy with LMB-100 in mice. Although tumor volume regressed significantly in combination-treated mice in comparison to

Table 1 Summary of IC_{50} (ng/mL) Values for MSLN-targeted RITs

Cell Line	LMB-12	LMB-100	LMB-164	LMB-11
KLM-1	0.17 ± 0.08	0.55 ± 0.06	0.38 ± 0.08	>1000
SW48	0.37 ± 0.15	0.78 ± 0.18	0.55 ± 0.03	>1000
HTB39	2.73 ± 0.5	2.71 ± 0.60	5.68 ± 0.64	>1000

Abbreviations: IC_{50} = half maximal inhibitory concentration; MSLN = mesothelin; RIT = recombinant immunotoxin therapy.

Figure 3 Female Nude Mice Were Inoculated Subcutaneously With SW48 Cells in the Right Flank and Underwent Treatment Beginning 7 Days Post Inoculation (Plotted as Day 0). Each Data Point Represents the Average Tumor Volume for n = 8 Animals Treated With the Indicated Recombinant Immunotoxin Therapy or Phosphate-buffered Saline Control. There Is a Significant Difference Between Immunotoxin-treated Tumors and Control Tumors ($P < .05$) Beginning on Day 11 (Indicated by *)

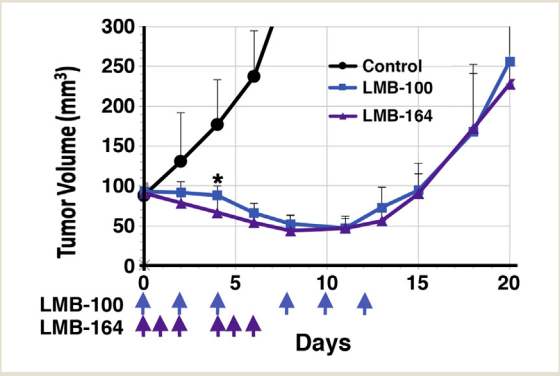
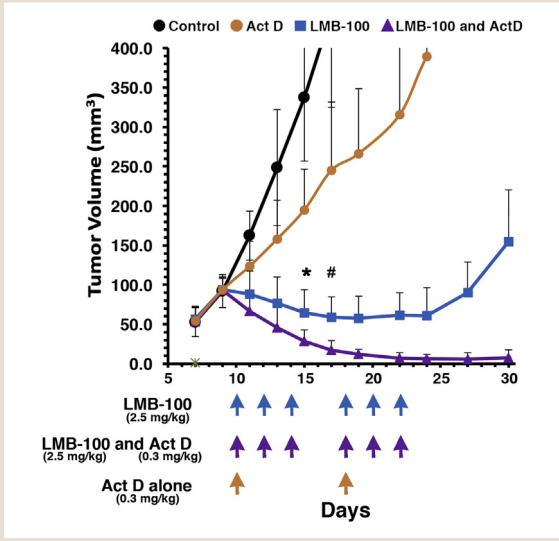


Figure 4 Female Nude Mice Were Inoculated Subcutaneously With SW48 Cells in the Right Flank and Underwent Treatment Beginning 10 Days Post Inoculation (Plotted as Day 10). Each Data Point Represents Average tumor Volume for n = 22 Animals Treated With LMB-100, actinomycin D, Combination of Actinomycin D and LMB-100, or Phosphate-buffered Saline Control. There Is a Significant Difference Between Groups Treated With Recombinant Immunotoxin Therapy (RIT) Monotherapy and Combination RIT-actinomycin D Therapy ($P < .05$) Beginning on Day 15 (Indicated by *). Actinomycin D Combination Therapy Is Significantly More Effective than RIT Monotherapy ($P < .05$) Beginning on Day 17 (Indicated by #)



Abbreviation: Act D = actinomycin D.

those undergoing vehicle treatment or oxaliplatin alone, there was no difference in tumor volume between RIT alone versus combination therapy (Figure 6). Both the combination and RIT monotherapy group experienced reduction to 48% of the initial tumor volume and reached 300 mm^3 tumor volume at 30 days in comparison to 6 days for vehicle treatment and 8 days for oxaliplatin alone. Furthermore, there were no complete regressions in the treatment groups (Figure 6).

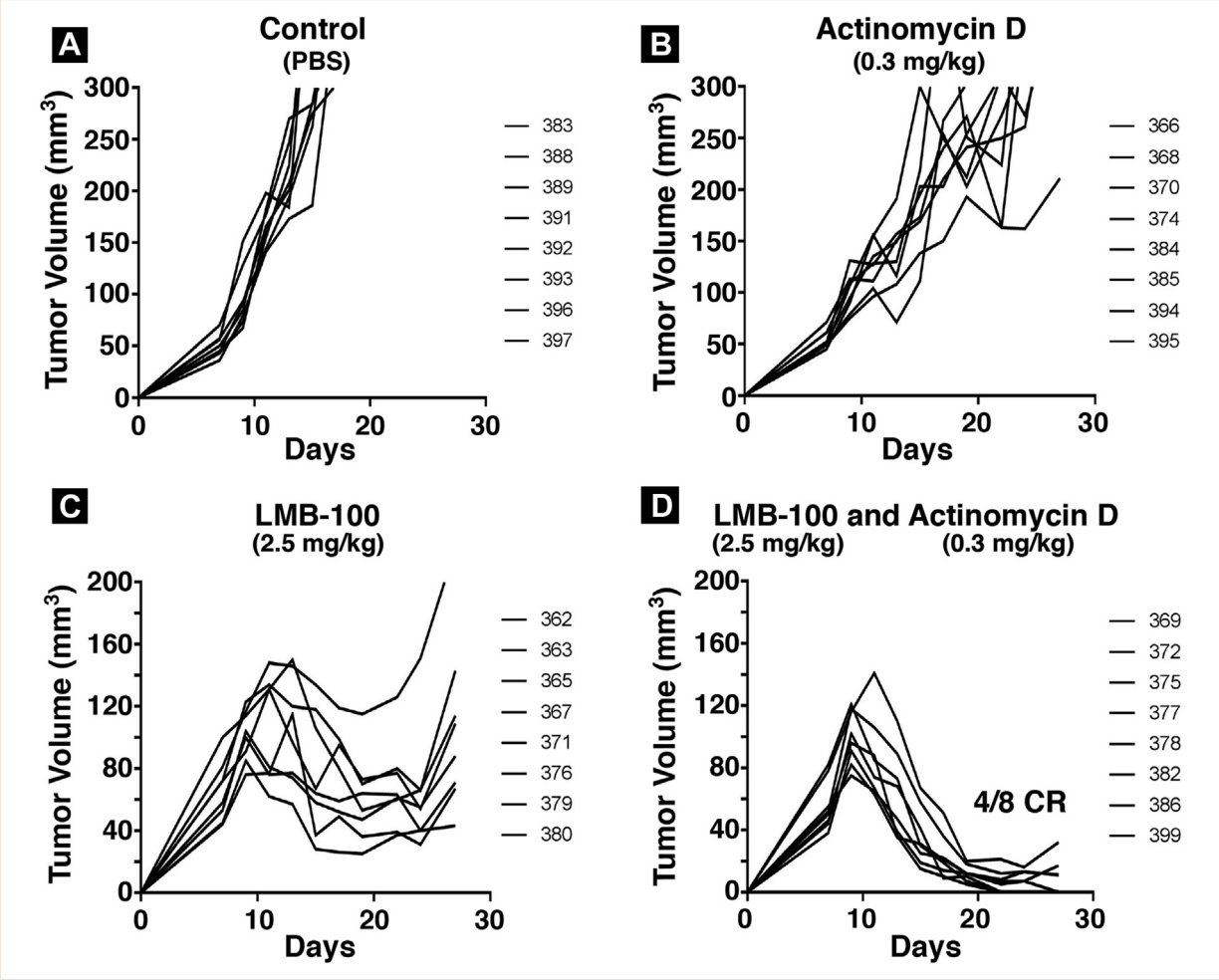
Discussion

MSLN-targeted RIT therapy with SS1P has produced tumor regressions in patients with mesothelioma in some patients with epithelioid mesothelioma, and its current applications are being tested in phase II trials at the NCI in various MSLN-expressing gastrointestinal malignancies.[11] Until recently, CRC was assumed to be a relatively poor target for this therapy.[15] However, with the advent of more specific antibody testing for membranous MSLN expression, malignancies once thought to be immune to RIT therapy may in fact be susceptible.[16] Given these findings, we attempted to create and explore MSLN-targeted RIT therapy in CRC. In vitro cytotoxicity experiments showed that not only were MSLN-expressing CRC cell lines susceptible to RIT therapy, but these cell lines responded to the same concentrations previously shown efficacious in KLM-1 pancreatic adenocarcinoma despite having a lower membranous expression of MSLN.

In vivo studies echoed these findings, with over 50% tumor regression in SW48 CRC tumors treated with LMB-100 monotherapy. In KLM-1 pancreatic cells, LMB-164 (an ABD containing RIT) was able to produce complete regressions at the concentrations tested in our study.[13] However, CRC cell lines were less susceptible, responding with tumor regression that was not statistically different than that of those tumors treated with LMB-100. The reason for the difference in response between these 2 cell lines is unclear at this time. It has been previously shown that MSLN shed from the membrane of tumor cells can have a sink effect, lowering the amount of RIT that is able to bind at the tumor cell membrane and halt protein uptake.[21] Whether or not CRC cells shed MSLN at a higher rate than those of pancreatic adenocarcinoma is unclear at this time and warrants further investigation. Another possibility lies in the fact that tumor stroma plays a role in inhibiting RIT diffusion into the tumor to malignant cell membranes, although the tumor stroma is also dense in pancreatic adenocarcinoma.[22]

Given these limitations, combination chemotherapy and RIT therapy warrants exploration. In fact, the unique mechanism of binding to EF-2 and halting protein synthesis of RITs provides a novel ability to reach both quiescent and active tumor cells. In our current study, we first explored the potential for combination with a current chemotherapeutic used in CRC therapy in oxaliplatin. Oxaliplatin, a platinum-based chemotherapy and DNA binding agent, has been previously shown to have additive or synergistic effects with fluorouracil and irinotecan in CRC in vitro and in vivo models.[23,24] Furthermore, prior synergy screens performed in our lab showed moderate combination activity between cisplatin and MSLN-targeted RIT.[18] Oxaliplatin has also been purported to

Figure 5 Spider Plots of Individual Tumor Sizes Per Treatment Group From a Representative Tumor Experiment. A, Control Group Treated With PBS. B, Mice Treated With 0.3 Mg/kg Actinomycin D. C, Mice Treated With 2.5 Mg/kg LMB-100. D, Mice Treated With 0.3 Mg/kg Actinomycin D and 2.5 Mg/kg LMB-100. The Combination-treated Group Was Noted to Have a 50% Complete Response Rate With a Significant Delay in Tumor Regrowth to 300 mm^3 Following Initial 2 Cycles of Therapy



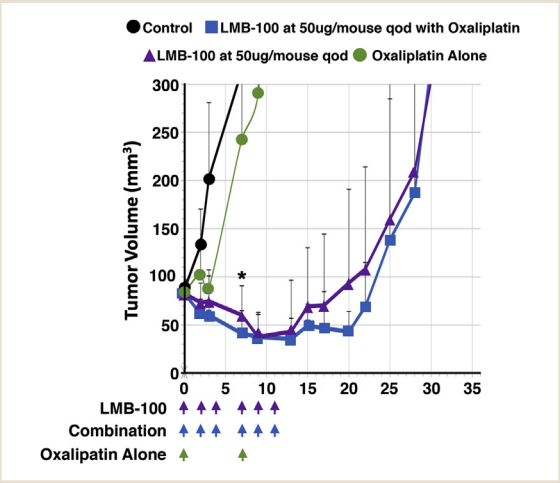
Abbreviations: CR = complete response; PBS = phosphate buffered-saline.

induce immunogenic cell death and activate the extrinsic pathway of apoptosis in CRC tumor cells in prior in vivo studies.[25,26] However, our current testing in SW48 cells displayed no added benefit outside of slight growth delay in the combination of these 2 drugs. This may be owing to the inability to achieve high enough in vivo concentrations of oxaliplatin to induce synergy with the RIT before first inducing toxicity.

Although actinomycin D is a chemotherapeutic agent largely used in the treatment of pediatric sarcoma, prior studies have shown an ability to synergize in vivo with RIT therapy in pancreatic cancer models.[18] Actinomycin D has also been shown to activate the extrinsic pathway of apoptosis when combined with immunotoxin therapy in vitro.[18] These findings led us to test actinomycin D in combination with MSLN-targeted RIT in our CRC model. Results from these experiments displayed not only significant synergy and tumor regression in vivo when compared with RIT monotherapy, but also showed 50% complete regression in tumors treated with this combination. Furthermore, repeat studies have shown that a small percentage of these tumors exhibit durable complete regression without the need for further treatment following an initial 2 cycles. This combination also proved safe at target dosing with less than 10% decrease in weight of mice treated during the described experiments.

Although the function of MSLN expression in malignancy has yet to be fully determined and a large-scale study of the clinical and mutational implications of MSLN expression in CRC still needs to be performed, clinical studies in pancreatic and ovarian cancer have shown that those tumors expressing MSLN tend to metastasize more readily and aggressively than their counterparts.[27-30] MSLN has also been implicated in the spread of ovarian cancer to the peritoneum through its interaction with MUC16.[31] MSLN expression has also been associated with a higher rate of KRAS mutation in lung cancer.[32] RIT therapy targeting MSLN plays a role in treating such malignancies that are not candidates for surgical

Figure 6 Female Nude Mice Were Inoculated Subcutaneously With SW48 Cells in the Right Flank and Underwent Treatment Beginning at 7 Days Post Inoculation (Plotted as Day 0). Each Data Point Represents the Average Tumor Volume for n = 8 Animals Treated With LMB-100, Oxaliplatin, Combination Oxaliplatin and LMB-100, or Phosphate-buffered Saline Control. There Is a Significant Difference Between Groups Treated With Recombinant Immunotoxin Therapy (RIT) Monotherapy or Combination RIT-Oxaliplatin Therapy ($P < .05$) Beginning on Day 12 (Indicated by *) and Persisting Throughout the Experiment. There Is No Significant Difference Noted Between Combination Therapy or Combination RIT Therapy



resection or do not respond to standard of care chemotherapeutics. Furthermore, the combination of RIT therapy and chemotherapeutics with novel mechanisms of action such as actinomycin D are another potential strategy to treat aggressive and refractory malignancies.

Conclusions

In conclusion, MSLN-targeted RIT therapy has a role in CRC that has previously been overlooked; and in combination with actinomycin D, can produce significant tumor regression including complete and durable regression.

Clinical Practice Points

- According to recent statistics from the American Cancer Society, CRC is the third most common cancer diagnosed in both men and women in the United States. The current estimates for the number of new CRC cases in the United States for 2019 are 101,420 of colon cancer and 44,180 of rectal cancer. It is also the second most common cause of death owing to cancer in the Western world. It is expected to cause about 51,020 deaths during 2019. Despite recent progress on early detection and therapeutic advances, new treatment options are needed to reduce the mortality rate from this cancer.
- MSLN is a cell surface glycoprotein expressed at high level on many malignancies including pancreatic adenocarcinoma, stomach cancer, ovarian cancer, and epithelioid mesothelioma. Recent data has shown that MSLN is expressed at clinically relevant levels on the surface of CRC. MSLN-targeted RITs consist of an anti-MSLN Fv fused to the catalytic domain of PE.
- In a nude mouse model, MSLN-targeted RIT treatment of SW48 CRC tumors resulted in a significant decrease in tumor volume as a single agent and in combination therapy with actinomycin D. MSLN-targeted RIT resulted in > 90% tumor volume reduction with 50% complete regressions.
- In conclusion, MSLN-targeted RIT therapy has a role in CRC that has previously been overlooked, and in combination with actinomycin D, can produce significant tumor regression including complete and durable regression.

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Disclosure

The authors declare no conflict of interest.

Supplemental Data

Supplemental figures accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2019.06.006.

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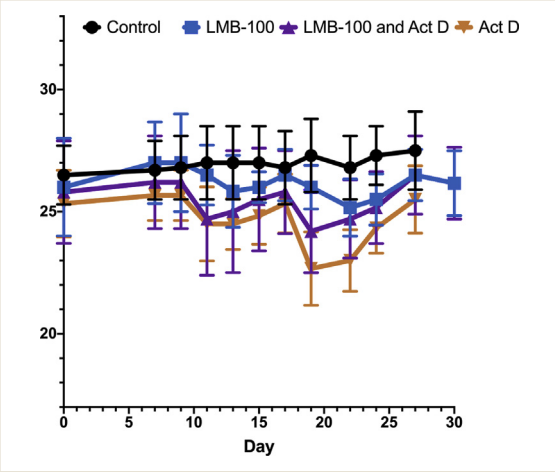
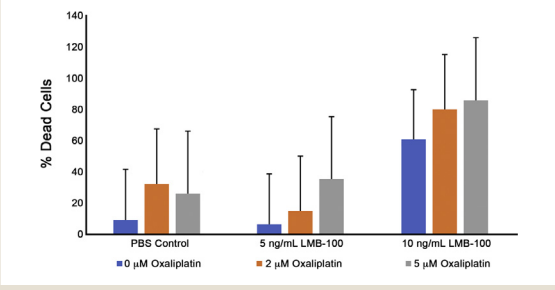
Supplemental Data

Supplemental Figure 1 Change of Body Weight of Mice in Various Treatment Group (n = 8 and Repeated 3 Times) Described in Figure 4



Abbreviation: Act D = actinomycin D.

Supplemental Figure 2 In Vitro Activity of Oxaliplatin in Combination of LMB-100 on SW-48 Cell Line (Repeated Twice)



Abbreviation: PBS = phosphate-buffered saline.