Phase I/II Study of the Mesothelin-targeted Immunotoxin LMB-100 with nab-paclitaxel for Patients with Advanced Pancreatic Adenocarcinoma

Running title: LMB-100 + NAB-paclitaxel in PDAC

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Abstract

Purpose: LMB-100 is a recombinant immunotoxin (iTox) consisting of a mesothelin-binding Fab for targeting and a modified *Pseudomonas* exotoxin A payload. Preclinical studies showed that combining taxanes with iTox results in synergistic anti-tumor activity. The objectives of this phase I/II study were to determine the maximum tolerated dose (MTD) of LMB-100 when administered with nanoalbumin bound (nab)-paclitaxel to patients with previously treated advanced pancreatic adenocarcinoma and to assess the objective response rate.

Patients and Methods: Patients (n = 20) received fixed dose nab-paclitaxel (125 mg/m² on Days 1 & 8) with LMB-100 (65 or 100 mcg/kg on Days 1, 3 & 5) in 21-day cycles for 1-3 cycles.

Results: Fourteen patients were treated on the dose-escalation and an additional 6 in the phase II expansion. MTD of 65 mcg/kg was established for the combination. Dose-limiting toxicity (DLT) resulting from capillary leak syndrome (CLS) was seen in 2 of 5 patients treated at 100 mcg/kg and 1 of 6 evaluable phase I patients receiving the MTD. Severity of CLS was associated with increases in apoptotic circulating endothelial cells (CECs). LMB-100 exposure was unaffected by anti-LMB-100 antibody formation in 5 of 13 patients during cycle 2. Seven of 17 evaluable patients experienced >50% decrease in CA 19-9, including 3 with previous exposure to nab-paclitaxel. One patient developed an objective partial response. Patients with biomarker responses had higher tumor mesothelin expression.

Conclusions: Although clinical activity was observed, the combination was not well tolerated and alternative drug combinations with LMB-100 will be pursued.
Translational Relevance

Mesothelin (MSLN) is a surface glycoprotein of unknown function with normal expression limited to mesothelial tissues. Over 90% of pancreatic adenocarcinomas express MSLN although it is not made in normal pancreas. LMB-100 specifically targets surface MSLN to deliver a novel toxin payload which halts cellular protein synthesis by irreversibly modifying and inactivating Elongation Factor-2. Pre-clinical data demonstrated synergistic anti-tumor activity of nab-paclitaxel with LMB-100. This study is the first to report on human testing of LMB-100. The tolerability, pharmacokinetics, immunogenicity and antitumor activity of the combination were investigated. Association between change in serum tumor marker and baseline tumor MSLN expression was also evaluated.
Introduction

Pancreatic cancer is an aggressive malignancy with a 5-year overall survival of only 9% despite recent advances in combination chemotherapy treatments (1-3). In fact, pancreatic cancer is now the 3rd most common cause of cancer death in the United States (4). Pancreatic ductal adenocarcinoma (PDAC) accounts for ~90% of this disease burden. Single agent immune checkpoint inhibitor treatments, targeted monoclonal antibodies, and therapies directed against receptor tyrosine kinases have been clinically ineffective for this disease except in rare cases (5). New research has begun to show that PDAC patients harboring BRCA mutations (~5-7%) may respond to PARP inhibition (6-9), but personalized therapies directed against tumor-associated mutations has been disappointing for most other PDAC patients as the most commonly mutated genes in PDAC (KRAS, TP53, DPC4 and CDKN2A) remain elusive drug targets (10). Novel therapies are needed to treat PDAC.

LMB-100 (previously called RG7787 and Ro6927005) is a second-generation iTox designed to kill cancer cells that express the cell surface glycoprotein mesothelin (MSLN). MSLN is a differentiation antigen for the mesothelial cells of the pleura, pericardium and peritoneum, but is also expressed by >90% of PDAC (11). LMB-100 consists of a humanized Fab that binds to MSLN with high affinity fused to a modified Pseudomonas exotoxin A (PE) payload (12). LMB-100 is directed to MSLN-expressing cells by the binding domain, then internalized through endocytosis, resulting ultimately in cytoplasmic release of PE. PE is an enzyme which kills cells by irreversibly modifying Elongation Factor-2 to halt protein synthesis, a unique mechanism of action. Pre-clinical studies demonstrated LMB-100 anti-tumor activity in mouse models of PDAC and other MSLN-expressing solid tumors (13,14). Phase I testing of
LMB-100 identified a single agent MTD of 140 mcg/kg. DLTs included capillary leak syndrome (CLS), and reversible elevations of creatinine.

Combination studies in the laboratory have identified synergistic anti-tumor effect when LMB-100 is combined with other anti-cancer drugs including dactinomycin (15), panobinostat (16) and taxanes (13,17). Complete responses were observed in a pancreatic tumor model when mice were co-treated with LMB-100 and nab-paclitaxel (17). Given these results, we conducted a clinical trial examining the safety and anti-tumor response of this combination in patients with PDAC.

Patients and Methods

Patients- Eligible patients were ≥ 18 years old and had a histologically confirmed diagnosis of PDAC. Advanced or recurrent disease previously treated with at least one line of standard chemotherapy was required. Prior nab-paclitaxel was permitted if > 4 months since last administration. Other requirements included: measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, adequate organ function including baseline documentation of LVEF ≥ 50% by echocardiogram and ambulatory oxygen saturation >88%. See full protocol in Supplementary Materials for full inclusion and exclusion criteria. The study was conducted in accordance with FDA regulations and Good Clinical Practice guidelines. The study protocol was approved by the National Cancer Institute (NCI) Institutional Review Board and written informed consent was obtained from all patients participating.
Study design and treatment- This open label, Phase I study of intravenous LMB-100 was conducted at NCI Center for Cancer Research (CCR) in Bethesda, MD (NCT02810418). Results for all study arms where patients received short infusion LMB-100 (30 minute infusion) with nab-paclitaxel (Arms A1 and A2) are reported here. This schedule of LMB-100 was given in prior single agent Phase I testing (NCT02317419, NCT02798536). Data pertaining to patients that received long infusion of LMB-100 (infusion >24 hours), an alternative administration schedule, as a single agent (Arm B1) or with nab-paclitaxel (Arm B2) will be reported separately. Arm B1 was enrolled concurrently with the A Arms (Supplemental Figure 1). Patients ineligible for Arm A or unwilling to receive nab-paclitaxel were enrolled on Arm B1. Following completion of Arm B1, enrollment on Arm A was halted pending full accrual of Arm B2. The primary endpoint for Arm A1, the Phase I dose escalation, was determination of a MTD for LMB-100 when given with nab-paclitaxel. For the Phase II expansion (Arm A2), objective response rate (ORR) by RECIST criteria was the primary endpoint. Secondary endpoints included assessment of adverse events (AEs), determination of pharmacokinetics, incidence of anti-drug antibodies (ADAs) against LMB-100 and change in tumor marker CA 19-9. The Phase II expansion was conducted using Simon minimax two-stage Phase II trial design to rule out an unacceptably low ORR of 5% ($p_0 = 0.05$) in favor of an improved ORR of 25% ($p_1 = 0.25$) with $\alpha = 0.10$ and $\beta = 0.10$. Planned enrollment would include 20 evaluable participants (including those treated at MTD on the phase I) through the second stage only if at least 1 of the first 13 participants experienced a response. Participants were considered evaluable for the Phase II if they had received at least one dose of LMB-100, had completed post-treatment imaging studies or were taken off-treatment for clinical progression of disease. The study was voluntarily closed
to accrual by the study team during the Phase II expansion after enrollment of 14 participants at MTD due to unacceptable toxicity.

A 3+3 design was used for the dose escalation. DLT was defined as most grade ≥ 3 non-hematologic toxicity (except for clinically insignificant electrolyte abnormalities) and specified severe hematologic toxicities (see Supplemental Materials for full protocol) that were related to LMB-100 and occurred within 21 days of the first LMB-100 administration. Nab-paclitaxel was administered at 125 mg/m² on days 1 and 8, and LMB-100 by 30-minute infusion as per dose escalation on days 1, 3 and 5 of each 21-day treatment cycle. This schedule was chosen to match the 21-day cycle length of prior LMB-100 single agent studies (NCT02317419, NCT02798536), to correspond with pre-clinical data on the efficacy of the regimen in mice (17), and to minimize interruptions in treatment due to myelosuppression from nab-paclitaxel in this heavily pre-treated patient population. On Day 1 (when both drugs were given), nab-paclitaxel infusion preceded LMB-100 by 30 minutes. This schedule was chosen because pre-clinical studies performed in mice demonstrated increased toxicity if nab-paclitaxel was infused immediately after LMB-100 or between the LMB-100 doses. All patients were pre-medicated with acetaminophen, diphenhydramine and ranitidine prior to LMB-100 infusion and ondansetron was available as needed for nausea. Initially, up to 4 treatment cycles were permitted, however, a 2-cycle limit was introduced by protocol amendment due to frequent incidence of infusion-related reaction (IRR) beyond cycle 2. Dose reductions of nab-paclitaxel were permitted as per package insert. LMB-100 used for this study was manufactured by Roche then transferred to NCI. Nab-paclitaxel was obtained from commercial sources.

Clinical Assessments- AEs were graded using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. AE of CLS was not routinely recorded. Instead, individual AEs
associated with CLS were each recorded separately from that designation to better understand the clinical symptoms associated. Baseline CT or MRI was performed within 28 days of treatment initiation with follow-up scans after every 2 cycles of treatment (~6 weeks). Tumor response was assessed per RECIST version 1.1. CA 19-9 tumor marker was drawn prior to treatment on the first day of each cycle and during follow-up visit after the last study treatment.

**Pharmacokinetic (PK) analyses**- LMB-100 drug concentrations were assessed from plasma drawn pre-dose, end of infusion (EOI), 1, 2, 3, 4 and 6 hrs post-EOI. Free LMB-100 plasma concentrations were measured with a validated ELISA with a lower limit of quantification (LLOQ) of 2.1 ng/mL. Testing was performed by contract with Frederick National Laboratory for Cancer Research operated by Leidos Biomedical Research, Inc. (Frederick, MD). For nab-paclitaxel assessment, blood samples were drawn pre-dose, 15 min post start of infusion, 5min prior to EOI, EOI, and 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-EOI. Briefly, total paclitaxel (bound to nab-paclitaxel particle, freely dissociated, and bound to plasma proteins) was measured by a validated ultra-high-performance liquid chromatography with tandem mass spectrometric (uHPLC-MS/MS) assay, as previously described (18) with one small modification to decrease the LLOQ from 10 ng/mL to 5 ng/mL. Briefly, 5x volume of acetonitrile was added to 100 µL of plasma containing nab-paclitaxel to precipitate proteins and release any bound paclitaxel. The mixture was vortexed and filtered via 0.2 µm Varian Captiva 96-well filtration plate. The supernatant was injected onto a Waters SymmetryShield® RP18 column, 2.1x50mm, 3.5µm before mass spectrometric detection and quantification of total paclitaxel plasma concentrations in the positive ion mode.

PK parameters, namely maximum plasma concentration (C\text{MAX}), area under the curve extrapolated to time infinity (AUC\text{INF}), half-life (T_{1/2}), clearance (CL), and volume of distribution
(Vz), were estimated using noncompartmental methods for both LMB-100 and nab-paclitaxel separately. Phoenix WinNonlin v8.1 (Certara, Cary, NC) was used to perform PK analyses; all plots and statistical tests were performed in GraphPad Prism, v8. The nonparametric Mann-Whitney test was used to compare group means for each parameter based on dose (65 mcg/kg vs 100 mcg/kg).

**ADA analysis**- A validated screening ELISA assay was applied to plasma samples drawn prior to drug administration on Day 1 of each cycle and at post-treatment follow-up visit. Samples with mean assay signals greater than the cut point (OD 0.05) were run in a confirmatory assay where a percent inhibition by spiked LMB-100 (32 µg/mL) was calculated. Inhibition of mean assay signal by >41.8% was used as threshold to define confirmed positives. Testing was performed by contract with Frederick National Laboratory for Cancer Research operated by Leidos Biomedical Research, Inc. (Frederick, MD).

**Assessment of circulating endothelial cells (CECs)**- Blood was collected in BD Vacutainer Cell Preparation Tubes (CPT) with sodium citrate (BD Biosciences, Franklin Lakes, NJ). PBMCs were isolated, aliquoted, and viably frozen and stored in liquid nitrogen until use. CEC analyses were performed using a MACSQuant flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously (19). Briefly, a minimum of $1 \times 10^5$ cells were acquired for each analysis. CECs were defined as negative for the hematopoietic marker CD45 (leukocyte common antigen), positive for the endothelial markers CD31 and CD146, and negative for the progenitor marker CD133. CECs were further sub-grouped into viable versus apoptotic populations. Viability was defined by the absence of 7-aminoactinomycin D (7-AAD) staining, and analysis was restricted to nucleated cells by gating on Hoechst 33342-positive cells. Data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR).
Statistical Analysis- Graphs were generated using Microsoft Excel or GraphPad Prism 7.01 and statistical analyses were performed in Prism or using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Spearman correlation was performed to demonstrate the association between change in percentage of apoptotic CEC and percent weight gain from baseline to cycle 1, day 5. A LOESS line was fit to these data to show their relationship. Error bars show standard deviations unless otherwise indicated. The safety analysis included all patients who received at least 1 dose of LMB-100.

Results

Patients- Twenty patients were enrolled to this study between August 2016 and February 2019 and assigned to receive short infusion LMB-100 (Arms A1 and A2) in combination with nab-paclitaxel. Fourteen were treated on the dose escalation (Arm A1) and six were part of the phase 2 expansion at MTD (Arm A2). Patients received a median of 2 cycles (range 1-3) of study treatment. Demographics, treatment history and disease burden of these patients are outlined in Table 1. Notably, 35% of patients had received prior nab-paclitaxel, 65% had liver metastases upon enrollment and 20% had clinically apparent ascites.

Establishing MTD of the combination (Arm A1)- Six patients received 100 mcg/kg LMB-100 (dose level 1) with nab-paclitaxel. Two of six patients developed DLT of myalgia (grade 3) with weight gain >5kg due to edema from CLS. Myalgia manifested as severe, narcotic-refractory pain that inhibited ambulation but was not associated with serum creatinine kinase elevation. MRI of the thighs in one of these patients showed fascial edema but no other abnormality of the
muscle to suggest myopathy or myositis. Other CLS-associated DLTs of fatigue (grade 3) and hypotension (grade 3) were also recorded in these 2 patients.

Since unacceptable toxicity was encountered at the first dose level, the LMB-100 dose was reduced to 65 mcg/kg (dose level -1). Eight patients were treated at this dose level. Two patients who did not experience DLT but required a hold or dose reduction of the day 8 nab-paclitaxel for reasons unrelated to LMB-100 treatment were replaced for the DLT analysis. Of 6 evaluable patients at this dose-level, CLS-associated DLTs of grade 3 edema and grade 3 urine output decrease were observed in 1 participant. The 65 mcg/kg dose was therefore advanced to Phase II testing.

Safety and tolerability (Arms A1 and A2)- Figure 1 shows AEs attributed to LMB-100. AEs experienced by those treated at the MTD of 65 mcg/kg on both the phase I and phase II are tabulated in composite. The most common adverse events were hypoalbuminemia (100%), edema (65%), fatigue (50%), hyponatremia (50%) and myalgia (45%). Grade 4 toxicities of ventricular dysfunction, hypotension and atrial fibrillation occurred in Patient #40 who was treated at the MTD on the Phase 2. Cycle 1 Day 5 LMB-100 was held in Patient #40. There were no other dose holds or dose reductions of LMB-100. Six patients discontinued treatment after Cycle 1 due to LMB-100 toxicity.

All AEs that were attributed to nab-paclitaxel are reported in Supplemental Figure 2. The most common events were decreases in blood counts (anemia, lymphopenia, leukopenia, neutropenia, thrombocytopenia). Fatigue, nausea and vomiting and diarrhea were also observed. These are consistent with the known toxicities of nab-paclitaxel. During cycle 1, 3 patients required hold of Day 8 nab-paclitaxel. Four patients required dose reduction of nab-paclitaxel during cycle 2 due
to prior neutropenia or worsening peripheral neuropathy. Severe AEs (grade 3-5) attributed to disease are reported in Supplemental Figure 3. All were associated with non-neutropenic infections.

**Manifestation of CLS**- CLS was the most common AE associated with LMB-100 treatment. All participants experienced at least one symptom related to CLS (Figure 2A). To better define the scope and timing of symptoms that patients with LMB-100-associated CLS experienced, we documented occurrence of each individual symptom rather than using the blanket *capillary leak syndrome* CTCAE term. Three participants were excluded from this analysis due to the development of sepsis requiring aggressive fluid resuscitation during Cycle 1, a treatment that confounded attributions. Hypoalbuminemia occurred in all participants and began by Day 3 (Figure 2B). Serum albumin continued to drop through Day 8 in most patients then began to recover after Day 15. Weight gain from fluid edema was observed in most participants (Figure 2C). Increases in weight began around Day 5 and peaked between Days 8 and 15. Nine of the 17 participants considered evaluable (53%) developed mild CLS, which was defined as CLS resulting in <5kg weight gain from edema (Figure 2A). AEs associated with mild CLS included hypoalbuminemia (grade 1 or 2), hyponatremia (grade 1), mild edema which resolved spontaneously, and asymptomatic hypotension. The remaining 8 participants (47%) developed significant CLS (grade 2-4, or not classified as CLS per CTCAE criteria) with weight gain of 5kg or more. Dizziness (4 of 8), tachycardia (4 of 8), edema requiring outpatient diuresis with oral furosemide (6 of 8), and hyponatremia (6 of 8, including 3 with grade 3) were also observed in this population. Four of these participants developed serious CLS (grade 3 or 4) that required hospitalization for symptom management such as diuresis with intravenous furosemide for refractory edema or fluid resuscitation for hypotension. One patient developed life-threatening
CLS (grade 4) due to edema of the ventricular walls which decreased left ventricular ejection fraction (Figure 2D). The impairment in cardiac function caused worsening hypotension and cardiogenic shock requiring vasopressor support for ~7 days until CLS resolved. No patient-specific factors that could have predicted occurrence of these life-threatening AEs were identified. The trial was closed to accrual of new patients in response to this severe AE.

CLS induced by iTox is presumed to be caused by non-specific uptake of iTox by endothelial cells resulting in their direct damage. We analyzed CECs from patient blood samples drawn prior to treatment and within 24 hrs of the Cycle 1 Day 5 LMB-100 dose. We found a moderately strong correlation between increase in apoptotic CECs and severity of CLS as measured by percent increase in body weight following treatment (Figure 2E, r = 0.68, 95% CI: 0.27-0.88). There was no association between % change in viable CECs (Supplemental Figure 4) or baseline kidney function (Figure 2F) and severity of CLS.

PK analysis- There were 20 patients with nab-paclitaxel serum concentration data available. Mean nab-paclitaxel plasma concentration followed a biphasic disposition (Figure 3A), consistent with a prior report (20). Individual parameter estimates, along with a statistical summary based on sex were also determined (Supplemental Table 1). It is difficult to compare the $C_{\text{MAX}}$ and AUC parameters with previously published literature because a different dose of nab-paclitaxel was administered. However, the half-life, clearance and volume of distribution were very similar to results shown in previous PK analyses of nab-paclitaxel in humans (20).

LMB-100 plasma concentrations were available for all 20 participants and declined in a mono-exponential manner following a short 30-min IV infusion (Figure 3B). On C1D1, patients given 100 mcg/kg had significantly higher $C_{\text{MAX}}$ (p=0.012) and greater AUC (p=0.006) than
those given 65 mcg/kg, but without significant differences in dose-normalized $C_{\text{MAX}}$ and AUC, as well as half-life, distribution volume and clearance (Table 2).

**ADAs and relationship to plasma LMB-100 concentrations**- During Cycle 1, $C_{\text{MAX}}$ greater than 100 ng/mL was measured for all 20 participants (Figure 3C). A wide distribution was observed for this parameter at MTD with values ranging from 140 to 1538 ng/mL. We hypothesized that this variation could be due to the presence of pre-existing ADAs. Indeed, we found a modest inverse correlation between baseline ADA value pre-treatment and Cycle 1 $C_{\text{MAX}}$ (Figure 3D). During Cycle 2, 8 of 13 participants had $C_{\text{MAX}} > 100$ ng/mL (62%), but 5 had very low drug levels (Figure 3C). All 5 with low drug levels had a positive ADA screening test at baseline (Figure 3E). However, 3 of the 8 participants with $C_{\text{MAX}} > 100$ ng/mL also tested positive for ADAs before starting treatment. Moreover, 2 participants with positive ADA testing performed immediately pre-treatment on C2D1 also achieved $C_{\text{MAX}} > 100$ ng/mL for Cycle 2. While a negative ADA test was predictive of achieving good drug levels, a positive ADA test did not predict poor Cycle 2 drug levels. Post-Cycle 2 ADA values were available for 8 participants. Seven of 8 (88%) developed high titer ADAs after receiving 6 doses of LMB-100. This suggests that few patients would achieve good drug levels if more than 2 cycles of LMB-100 were administered.

**Anti-tumor activity**- CA 19-9 response was evaluable for 17 participants. Maximum decline in CA 19-9 of >50% was observed in 7 participants during their 2 cycles of treatment with LMB-100 and nab-paclitaxel (Figure 4A). Three of 7 with CA 19-9 response had received prior nab-paclitaxel. CA 19-9 normalized in patient #15. This patient also experienced a confirmed partial response with 100% decrease of a FDG-avid mass anterior to the liver (Figure 4B). Patient #15 continued to have imaging abnormalities consistent with tumor near the pancreatic head so could
not be classified as a complete responder. Additionally, patient #25 who had received prior nab-paclitaxel and had liver-predominant disease experienced a 17% reduction in tumor burden. These two participants with the best imaging responses both had Cycle 2 LMB-100 $C_{\text{MAX}} > 1000$ ng/mL.

**Association of anti-tumor activity with MSLN expression** - Immunohistochemistry (IHC) to assess tumor MSLN expression was performed on archival tumor samples. These samples were available for 10 of 20 participants, including 6 of the 7 with CA 19-9 response to treatment (Supplemental Table 2). All participants with CA 19-9 response had MSLN expression in 40% or more of their tumor cells (Figure 4A). The 4 participants with MSLN IHC data available and worst CA 19-9 responses expressed MSLN in 30% or less of their tumor cells. Data from this limited number of patients suggests that CA 19-9 response to combination treatment with LMB-100 and nab-paclitaxel may correlate with higher tumor MSLN expression.

**Discussion**

Our data show that the combination of nab-paclitaxel with LMB-100 may have anti-tumor activity but amplifies the toxic side effects of the immunotoxin. The combination caused severe symptoms of CLS even when LMB-100 was administered at less than 50% of the single agent MTD. In addition, unique manifestation of CLS were observed when LMB-100 was combined with nab-paclitaxel such as severe myalgia and cardiac toxicity. These severe toxicities outweighed the benefits of treatment with this combination.

Here, we have reported detailed clinical information on the manifestation of immunotoxin-associated CLS. This syndrome begins with albumin loss occurring within 48
hours of iTox infusion, followed by weight gain from edema that peaks by 2 weeks into the cycle. All CLS-related toxicities were fully reversible with the administration of adequate supportive care. No long-term changes in renal or cardiac function were observed. The etiology of iTox-induced CLS is not well understood. Early generation iTox’s targeting Lewis antigens resulted in CLS at least in part due to specific uptake of iTox by Lewis antigen-expressing endothelial cells. However, for iTox like LMB-100 that target a protein not expressed by endothelial cells another mechanism must be responsible. While non-specific uptake of LMB-100 by endothelial cells is possible, in vitro testing using cultured endothelial cell lines has shown that exposure to concentrations of iTox that far exceed our observed C<sub>MAX</sub> is insufficient to induce cell killing unless the cells express the iTox target (21). Nevertheless, we found that apoptotic CECs increased in all patients immediately following LMB-100 treatment. Further, a quantitative association between severity of CLS and numbers of apoptotic CECs was observed. Further study will be required to determine whether this is due to off-target uptake of LMB-100 by endothelial cells causing endothelial cell killing or whether a secondary process such as iTox-induced inflammation produces this damage. Additionally, we do not know whether co-administration of nab-paclitaxel is necessary to trigger the observed increase in apoptotic CECs.

While the combination of nab-paclitaxel with LMB-100 will not be pursued further, other clinical trials testing LMB-100 are planned or continue (NCT03644550). Several of our observations pertaining to companion diagnostics are relevant to these studies. First, our data have shown that patients with the best CA 19-9 response to LMB-100/ nab-paclitaxel tested positive for MSLN expression in ≥40% of tumor cells in archival tumor samples, while non-responders all had expression less than this. Secondly, we have found that all patients with a negative ADA test achieved expected circulating LMB-100 levels during second cycle treatment.
Thirdly, a positive ADA test did not guarantee poor circulating LMB-100 levels during cycle 2. These data provide an initial indication of which patients are most likely to benefit from LMB-100 if corroborated by additional patient data.

In summary, the LMB-100 and nab-paclitaxel combination did show evidence of clinical activity but was too toxic for patients with pancreatic cancer. Future LMB-100 combination studies with other drugs are planned.

Conclusions

ADAs against LMB-100 limit the amount of drug that can be given. Toxicity of this combination makes it unsuitable for further development.

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References


## Figure Legends

### Table 1. Baseline Patient Demographics and Clinical Characteristics

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<tr>
<td><strong>No. of patients</strong></td>
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<tr>
<td>Arm A1</td>
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<tr>
<td>Dose Level 1 (100 mcg/kg LMB-100)</td>
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<td>Dose Level -1 (65 mcg/kg LMB-100)</td>
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<td>Median CA19-9 (range), in U/mL</td>
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Table 2. PK Summary for LMB-100

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<td>1282 (14.9)</td>
<td>1306</td>
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<td>AUCinf (hr*ng/mL)</td>
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<td>2170</td>
<td>1409 (47.9)</td>
<td>n/a</td>
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<td></td>
</tr>
<tr>
<td>AUCinf/D (hr*ng/mL/mg)</td>
<td>415 (56.5)</td>
<td>301</td>
<td>315 (45.8)</td>
<td>n/a</td>
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<td></td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>1.25 (51.3)</td>
<td>0.98</td>
<td>0.99 (12.9)</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/hr)</td>
<td>2.97 (44.3)</td>
<td>3.32</td>
<td>4.98 (116)</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vd (L)</td>
<td>4.91 (20.5)</td>
<td>4.75</td>
<td>7.48 (106)</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values expressed as arithmetic mean (%CV)

1 Eight patients were given dose level -1 on Arm A1, but patient 1017 did not have enough data to calculate an elimination rate, hence AUCinf, CL or Vd

2 Three of eight patients on Arm A1 dose level -1 either didn’t have C2 data or had no measurable LMB-100 by C2D1 (patients 1010, 1028); no patients have sufficient data to estimate elimination rate, hence AUCinf, CL, or Vd

3 Of the 6 patients on Arm A2, three had data for C2D1, but none had enough to calculate an elimination rate, hence AUCinf, CL, or Vd
Figure 1

All AEs of grade ≥2 attributed to LMB-100. Maximum grade reported by each participant is recorded. AE’s that never exceeded Grade 1 in any participant are shown if reported by at least 2 participants. *Electrolyte disturbance* includes CTCAE criteria: hypokalemia, hypocalcemia and hypercalcemia. *GI disturbance* includes: abdominal pain, distension and bloating. *Elevated LFTs* include: ALT and/or AST elevation.

Figure 2

Clinical picture of CLS. (A) Spectrum of CLS-related AEs reported for each evaluable participant. (B) Time-scale for development of CLS-associated symptoms of hypoalbuminemia and (C) weight gain from fluid edema. (D) Pre- and post-treatment echocardiogram images record acute change in ventricular wall thickness, and (E) Change in percentage of apoptotic circulating endothelial cells (CECs) pre- versus post-treatment (drawn within 24 hrs of completing Cycle 1 LMB-100 treatment on Day 5 or 6) was plotted against percent change in patient body weight, an estimate of CLS severity (Spearman coefficient \( r = 0.68, 95\% \text{ CI: } 0.27-0.88, n = 16 \)). Values indicated on the axes are median, 10th and 90th percentiles. A LOESS line is included to help interpret the association. (F) Relationship between baseline Creatinine Clearance (CrCl) as calculated by Cockcroft-Gault estimation and percent change in patient body weight. No statistically significant correlation was observed.

Figure 3

Systemic drug concentrations and relationship to ADA formation. (A) Mean serum nab-paclitaxel concentration. (B) Mean plasma LMB-100 concentrations for Cycle 1 Day 1. (C)
Variations in LMB-100 C<sub>MAX</sub> by patient and cycle. (D) Relationship between presence of pretreatment ADAs against LMB-100 and LMB-100 C<sub>MAX</sub> during Cycle 1. (E) ADA measurements pre-treatment are indicated by each plotted point for participants receiving 65 mcg/kg (circles) and 100 mcg/kg (squares). Solid connecting lines indicate participants with good cycle 2 LMB-100 plasma concentration (max > 100 ng/mL), while black hatched lines with filled markers indicate poor cycle 2 LMB-100 plasma concentrations. Cycle 2 LMB-100 concentration was not available (N/A) for some participants (gray dotted line). Binomial ADA test read-out is shown in pale gray.

**Figure 4**

Anti-tumor activity of LMB-100 and nab-paclitaxel. (A) Bars show maximum change in tumor marker CA 19-9 during treatment. The percent of tumor cells expressing MSLN in archival tumor tissue (when available) as determined by a pathologist blinded to treatment response is shown in blue. See Supplemental Table 2 for tabulation of tumor MSLN expression in all study participants. (B) CT imaging showing confirmed PR of Patient #15. PET-avid mass anterior to liver (indicated by arrow) resolved under treatment then began to regrow by 13 weeks post-treatment (Tx).
Figure 2

A

<table>
<thead>
<tr>
<th>Patient #</th>
<th>100 mcg/kg</th>
<th>65 mcg/kg</th>
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<tbody>
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<td>weight gain ≤5 kg</td>
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<td>LV dysfunction</td>
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</tbody>
</table>

B

Serum Albumin (g/dL) vs. Day of Cycle 1

C

Change in Weight (kg) vs. Day of Cycle 1

D

Baseline

E

Change in apoptotic CECs (%) vs. % weight gain

F

C Cr (mL/min) vs. % weight gain

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Figure 4

A

Intensity of MSLN staining: 1+ ☛ 2+ ☛ 3+

% MSLN(+) tumor cells

Change in CA19-9 (%)

prior NAB-p ☘️ 100 mcg/kg ☛ 65 mcg/kg

Patient No.

B

Baseline  ☉  3w post-Tx ☉  8w post-Tx ☉  13w post-Tx

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Clinical Cancer Research

Phase I/II Study of the Mesothelin-targeted Immunotoxin LMB-100 with nab-paclitaxel for Patients with Advanced Pancreatic Adenocarcinoma

Christine Alewine, Mehwish Ahmad, Cody J. Peer, et al.

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