Abbreviated Title: LMB-100 in Mesothelioma
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Title: A Phase I Study of the Mesothelin-Targeted Immunotoxin LMB-100 with or without *Nab*-Paclitaxel (Abraxane) in Patients with Malignant Mesothelioma

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Investigational Agent:

Drug Name:	LMB-100 (formerly RO6927005)
IND Number:	152907
Sponsor:	Center for Cancer Research, NCI, NIH
Manufacturer:	F. Hoffmann-La Roche

Commercial Agent

Nab-paclitaxel

PRÉCIS

Background:

- Although mesothelioma patients with a limited tumor burden may benefit from surgical resection, most patients have advanced disease at diagnosis and are not candidates for cytoreductive surgery.
- For mesothelioma patients who are not eligible for curative surgery, the median survival with supportive care alone is 6 months whereas with the current standard treatment, a combination of cisplatin and pemetrexed, the median survival is 12 months.
- Mesothelin, a tumor differentiation antigen, is expressed in over 95% of epithelioid mesothelioma. Mesothelin is a suitable candidate for targeted therapy due to its very limited expression in normal human tissue and its high expression in several tumors including mesothelioma.
- LMB-100 is a novel recombinant anti-mesothelin immunotoxin developed for the treatment of patients with solid tumors that express mesothelin. Mesothelin is targeted by linking a humanized fragment of the anti-mesothelin Fab to a de-immunized Pseudomonas exotoxin (PE)
- The clinical use of first generation immunotoxins such as SS1P was hampered mainly by their high immunogenicity. LMB-100 is a next generation PE-fusion protein that has been protein-engineered to maximally reduce its immunogenicity. LMB-100 has shown broad activity against different mesothelin-expressing cancer cell lines and tumor xenograft models.
- The combination of LMB-100 plus *nab*-paclitaxel has shown anti-tumor efficacy in different mesothelioma patient derived xenograft (PDX) models compared to LMB-100 or *nab*-paclitaxel alone.

Objectives:

- To identify the recommended phase 2 dose (RP2D) of LMB-100 +/- *nab*-paclitaxel in patients with treatment refractory advanced epithelioid or biphasic mesothelioma and evaluate potential efficacy of the identified RP2D.

Eligibility:

- Age $\geq$ 18 years
- Histologically confirmed advanced pleural or peritoneal mesothelioma
- Subjects must have had at least 1 prior chemotherapy regimen with last dose of previous therapy occurring at least 3 weeks before the start of study treatment
- Subjects enrolling in Arms B1 and B2 must not have received paclitaxel or *nab*-paclitaxel within 4 months prior to initiation of study therapy
- Adequate organ function
- Participants with CNS metastases or prior pneumonectomy are excluded

Design:

- This is a Phase I, open-label study to evaluate the safety, pharmacokinetics, and activity of LMB-100 +/- *nab*-paclitaxel in patients with treatment refractory advanced epithelioid or biphasic mesothelioma.

- Subjects in Arms A1 and A2 (completed) will receive LMB-100 monotherapy; subjects in Arms B1 and B2 will receive LMB-100+*nab*-paclitaxel
- LMB-100 will be administered intravenously on days 1, 3 and 5 of each 21 day cycle for up to 4 cycles in Arms A1 and A2 and up to 2 cycles in Arms B1 and B2
- *Nab*-paclitaxel (Arms B1 and B2 only) will be administered intravenously on days 1 and 8 of each 21 day cycle for up to 6 cycles
- Tumor response will be assessed after every 2 cycles
- Optional tumor biopsies may be collected at baseline and after 2 cycles of therapy
- The accrual ceiling will be set at 34

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective:

- To identify the recommended phase 2 dose (RP2D) of LMB-100 +/- *nab*-paclitaxel in patients with treatment refractory advanced epithelioid or biphasic mesothelioma.

1.1.2 Secondary Objectives

- To define the pharmacokinetics characteristics of LMB-100 +/- *nab*-paclitaxel, including the number of effective cycles of LMB-100 therapy that can be given before the development of anti-LMB-100 antibodies
- To assess the safety and tolerability of LMB-100 +/- n*ab*-paclitaxel in patients with treatment refractory epithelioid or biphasic mesothelioma
- To determine the duration of response, progression free survival and overall survival
- To determine the efficacy at the RP2D of LMB-100 +/- *nab*-paclitaxel with respect to objective response rate in patients with treatment refractory advanced epithelioid or biphasic mesothelioma.

1.1.3 Exploratory Objectives:

- To establish the correlation of response to treatment with tumor mesothelin expression;
- To evaluate changes in the tumor microenvironment following treatment with LMB-100 +/- *nab*-paclitaxel
- To identify the mechanism for Pseudomonas exotoxin-mediated capillary leak syndrome
- To investigate the potential of soluble mesothelin levels and levels of megakaryocyte

potentiating factor to predict any therapeutic response

1.2 BACKGROUND AND RATIONALE

1.2.1 Background on Mesothelioma

Mesothelioma is a neoplasm originating from the mesothelial cells lining human body cavities. Mesothelioma may involve the pleura and less frequently, the peritoneum. Approximately 3000 new cases are diagnosed every year in the US alone. The epithelioid variant is the most common, comprising about 60 percent of all mesotheliomas. Malignant pleural mesothelioma is an aggressive disease with poor prognosis. Although patients with a limited tumor burden may benefit from surgical resection, most patients have advanced disease at diagnosis and are not candidates for cytoreductive surgery.[1] For patients who are not eligible for curative surgery, the median survival with supportive care alone is 6 months whereas with the current standard treatment, a combination of cisplatin and pemetrexed, the median survival is 12 months.[2]

Peritoneal mesothelioma represents about one-fifth to one-third of all forms of mesothelioma; there are approximately 400 new cases in the United States each year.[3] Cytoreductive surgery and hyperthermic perioperative chemotherapy is the accepted initial management for suitable patients with peritoneal mesothelioma.[4-7] Peritoneal mesothelioma patients with surgically unresectable disease or whose medical co-morbidities preclude surgery are considered for palliative systemic therapy. Due to its relatively low incidence and inherent difficulties of radiologic assessment, few studies of systemic therapy have been conducted. Treatment recommendations are often extrapolated from pleural mesothelioma and outcomes are poor.

1.2.2 Mesothelin as a target for cancer therapy

Mesothelin is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein, which is present in a restricted set of normal adult tissues, such as the mesothelial lining of the pleura and pericardium. Immunohistochemistry has shown that mesothelin is highly expressed in nearly all epithelioid mesotheliomas as well as epithelial components of biphasic mesothelioma in addition to pancreatic ductal adenocarcinomas and in a high percentage of epithelial ovarian cancers and non-small cell lung cancer (NSCLC).[8] Although the normal biological function of mesothelin is unknown, growing evidence suggests that it may play a role in tumorigenesis and metastasis. Its limited expression in normal human tissue and high expression in tumor makes mesothelin an excellent target antigen for antibody-based immunotherapy.[9]

Because of the high expression of mesothelin in many malignancies, a variety of agents are being developed to target mesothelin. Results of several ongoing clinical trials of immunotherapy agents directed against mesothelin have shown that targeting mesothelin is safe and does not result in toxicity to essential normal tissues. Both antibody-based therapies, as well as mesothelin vaccines, are being investigated.[10] The Laboratory of Molecular Biology (LMB) and the Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute have pioneered the use of mesothelin- targeted agents and clinical trials over the last decade.

1.2.2.1 Recombinant Immunotoxins (RITs)

1.2.2.1.1 Mechanism and structure of RITs

RITs are antibody-based therapeutics that carry a toxin payload. RITs that target mesothelin contain a genetically engineered variant of Pseudomonas exotoxin A (PE) in which the native cell-

binding domain of PE is replaced by a mesothelin-binding antibody fragment. In this way, the RIT binds specifically to mesothelin on the cell surface and gets internalized through endocytic processes. In the cytosol, PE catalyzes an irreversible, inactivating modification of eukaryotic Elongation Factor-2 (eEF-2). This prevents the elongation step of protein synthesis, halting production of new cellular proteins, a stressor that triggers apoptosis. This mechanism of action results in cytotoxicity to both proliferating and non-dividing cells, therefore proper targeting is critical to the safety profile.[11]

Tumor-targeted bacterial toxins that inhibit the protein synthesis of cancer cells have previously shown signs of preclinical and clinical efficacy. For instance, SS1P, a classical immunotoxin format molecule consisting of the mouse anti-mesothelin Fv, SS1 and a 38 kDa PE toxin fragment (PE38) is one example. Besides SS1P, the most clinically advanced PE-based immunotoxins are moxetumomab pasudotox, a CD22-targeted fusion protein in development by LMB/Astra Zeneca/ Medimmune, and LMB2, a CD25-targeted PE fusion protein developed by LMB. Both agents have shown encouraging signs of efficacy in different hematological malignancies and are currently in Phase II and III clinical trials. Denileukin difitox is the only immunotoxin approved by the US Food and Drug Administration (FDA); a fusion protein of diphtheria toxin with interleukin-2 that is clinically used for intravenous treatment of cutaneous T cell lymphoma.

1.2.2.1.2 SS1P - a recombinant immunotoxin targeting mesothelin

The first mesothelin directed agent to enter the clinic was the recombinant immunotoxin (RIT) SS1P. The safety and tolerability of SS1P was determined in two phase I clinical trials in patients. In one trial SS1P was given as a bolus infusion over 30 minutes and the dose-limiting toxicity was pleuritis.[12] In another trial SS1P was given as a continuous infusion for 10 days. Dose-limiting side effects were very similar to the bolus infusion trial.[13] In neither trial were major responses observed, although there was shrinkage of small volume disease in some patients. Phase I studies established a maximum tolerated dose (MTD) of 45 mcg/kg for SS1P given every other day for three total doses per cycle. Dose limiting toxicities (DLTs) were pleuritis, an expected on-target off-tumor toxicity caused by SS1P-induced inflammation of the normal pleura, and secondly, vascular leak syndrome (VLS).

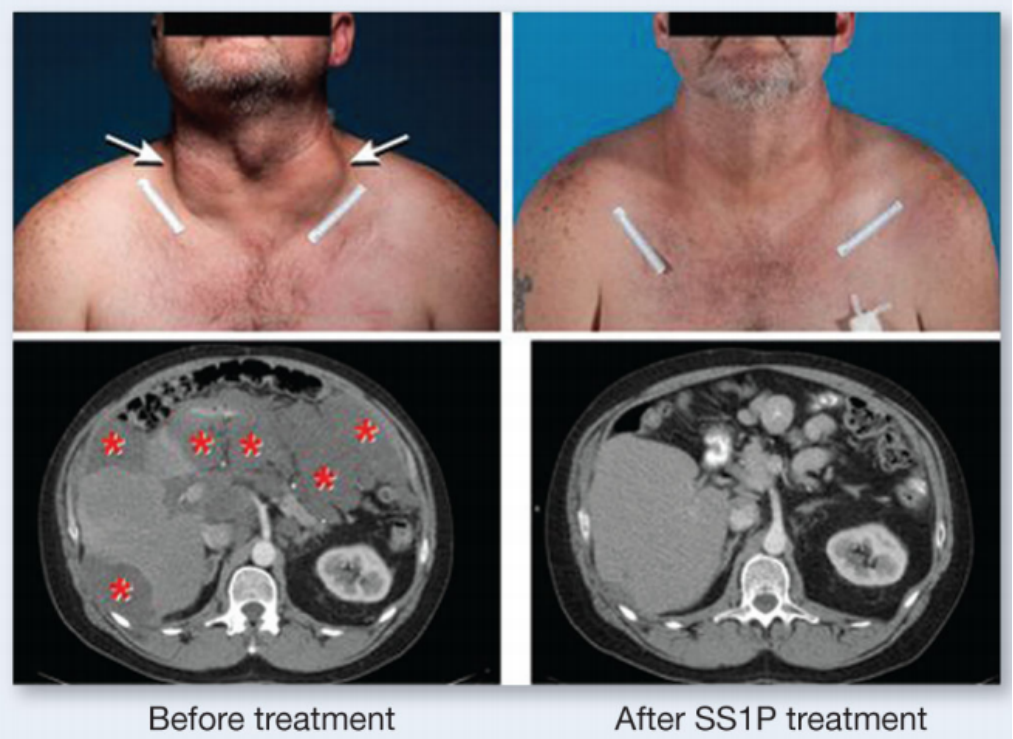
These early trials demonstrated that the efficacy of SS1P is limited by its immunogenicity. Anti-SS1P neutralizing antibodies (Nabs) formed after one cycle in ~90% of patients preventing effective drug exposure in subsequent cycle.[12, 14] Development of human antibody response to immunotoxins in patients with solid tumors has been a significant impediment to their clinical development and previous efforts to limit their immunogenicity by treatment with single agents such as steroids, cyclophosphamide, cyclosporine or rituximab have not been successful.[15-17] However, we have recently shown that when SS1P was administered with a lymphocyte-depleting conditioning regimen of pentostatin and cyclophosphamide, it delayed the development of anti-SS1P antibodies in immunocompetent mice.[18]

This strategy of using pentostatin and cyclophosphamide to prevent human antibody response to SS1P was evaluated in a pilot study of heavily pre-treated chemotherapy refractory mesothelioma patients. Eleven patients were enrolled in the study and received pentostatin and cyclophosphamide prior to SS1P administration. This regimen was well tolerated and no patient developed opportunistic infections. As predicted this regimen decreased the development of human antibodies to SS1P; only two of ten patients developed antibodies after cycle 1 of therapy, which was significantly better than in prior clinical trials where about 90% of patients developed antibodies after one cycle of therapy.[19] Remarkably, three of the ten evaluable patients with

extensive tumor burden had durable partial response and two of these patients had complete metabolic response by PET scan. All three patients were alive after 27, 25 and 22 months of starting therapy. In addition, two patients who did not initially respond to SS1P had a dramatic anti-tumor response when treated with chemotherapy to which they had not previously responded. SS1P plus pentostatin/cyclophosphamide regimen is currently being evaluated in more patients with mesothelioma. Major tumor shrinkage in the neck and abdomen of a patient with metastatic peritoneal mesothelioma treated with SS1P in combination with pentostatin and cyclophosphamide is shown in Figure 1.

These results provide a proof of principle that RITs can have meaningful clinical efficacy in patients with advanced solid tumors. A new generation of immunotoxin molecules with reduced immunogenicity and non-specific toxicity has been developed through protein engineering techniques.

Figure 1. Representative response to SS1P in a mesothelioma patient



1.2.3 LMB-100

1.2.3.1 Rationale for the development of LMB-100

The clinical use of SS1P, and of immunotoxins in general, has been hampered mainly by their high immunogenicity which limits the number of effective treatment cycles that patients can receive. LMB-100 (see Figure 2 for structure) is a next generation PE-fusion protein that has been protein-engineered to maximally reduce its immunogenicity by:

- Using a fully humanized Fab fragment derived from the anti-mesothelin antibody SS1 for tumor targeting

- Substituting the bulk of domain II (residues 251–273 and 284–394 of native PE) by an extended furin cleavable linker whose sequence is devoid of any T cell neo-epitopes
- Deimmunizing domain III of PE, which has the catalytic activity for ADP-ribosylation by introducing 7 point mutations that silence B- and T-cell epitopes

Classical PE-based immunotoxins, such as SS1P, contain a 38 kD fragment of the exotoxin encompassing the so-called translocation domain II and the catalytic domain III. Omission of the domain II from LMB-100 has not only removed a highly immunogenic 14 kD portion of PE that contains the main T-cell epitopes,[20] but has also resulted in reduced incidents of VLS in animal models of VLS.[21]

1.2.3.2 Development of LMB-100:

LMB-100 (previously RO6927005 and RG7787) is a next generation anti-mesothelin RIT developed in NCI's Laboratory of Molecular Biology in collaboration with Roche (Figure 2). LMB-100 contains a newly engineered PE fragment that has improved activity against most mesothelin-expressing cancer cell lines in vitro, and is also much less toxic than SS1P in pre-clinical models. This improved therapeutic window allows administration of three to eight times the dose of RIT to mice, rats and monkeys compared to SS1P. The new PE contains modifications specifically designed to reduce immunogenicity of the molecule. This includes deletion of a 14 kD sequence that precedes the catalytic domain and seven point mutations within the catalytic domain itself. These changes ablate the major human B cell epitopes within the molecule and also the most antigenic T cell epitope.[21, 22] The anti-mesothelin targeting region of LMB-100 uses a humanized Fab fragment instead of the smaller dsFv fragment used in SS1P. This increases molecular weight of the RIT above the threshold required to prevent filtration by the kidney and increases half-life.

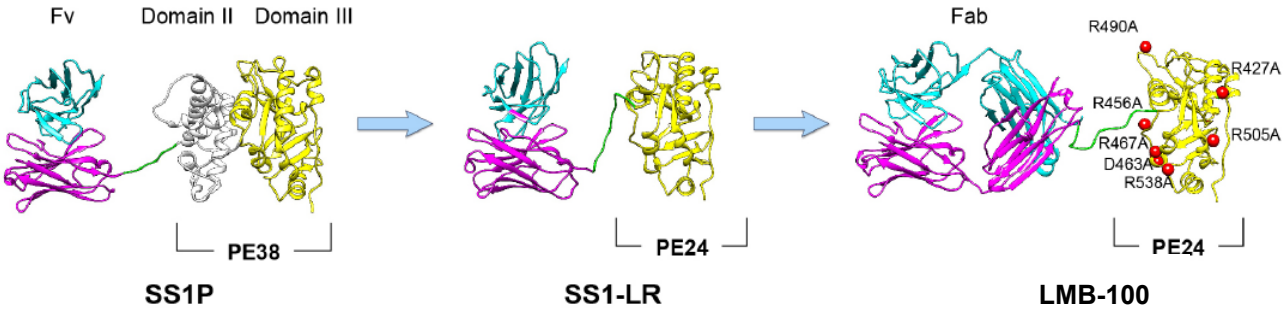


Figure 2. Structural models of SS1P and its de-immunized variants SS1P-LR and LMB-100 are shown.

The targeting domain consists of VL (cyan) and VH (magenta). The linker between the targeting domain and PE contains the furin cleavage site (green), which is required for toxin cytotoxic activity. The furin cleavage site is part of PE Domain II. The remainder of Domain II (gray) is unnecessary for cytotoxicity and has been deleted in the PE24-based toxins, SS1-LR and LMB-100. Domain III (yellow) is the catalytic domain of PE. In LMB-100, alanine point mutations were introduced at seven bulky hydrophilic residues (red) to silence human B cell epitopes within this domain. Deletion of Domain II reduces the size of the molecule into the range where it can be easily filtered by the kidneys, reducing serum half-life. LMB-100 contains a larger humanized Fab for targeting which raises its molecular weight above this threshold.

1.2.3.3 Nonclinical Studies

1.2.3.3.1 Nonclinical Pharmacology

In vitro LMB-100 inhibited viability of a variety of mesothelin-positive cancer cell lines at effective concentrations typically around 14 pM (~1 ng/mL). The cytotoxic potency of LMB-100 varied between 0.35 ng/mL in primary mesothelioma cells (RH21) and 15.7 ng/mL in an adenosquamous lung carcinoma cell line (H596). Binding studies showed that while the Fab fragment did not bind to mouse or rat mesothelin, the binding affinities to cynomolgus and human mesothelin were identical. In agreement with this, LMB-100 induced apoptosis in mesothelin-positive primary cynomolgus pericardial cells and significantly impaired viability of HEK293 cells transfected with human mesothelin, but not of rat mesothelin transfected or untransfected HEK293 cells. In addition, control experiments showed that free PE24 was 100–1000 fold less potent on mesothelin-positive target cell lines, confirming low cytotoxic potential of PE24 lacking a targeting moiety.

LMB-100 showed broad activity against different mesothelin-expressing cancer cell lines and patient derived xenograft models.[23, 24]

Animal studies demonstrated that a single cycle of LMB-100 treatment given at an optimal dose of approximately 2 mg/kg, 3 × per week, every other day (QOD) achieved tumor regressions in subcutaneous xenografts of adenosquamous lung carcinoma (H596) in severe combined immunodeficient (SCID) beige mice. Three consecutive treatment cycles, given with 1 week breaks in between, led to massive shrinkage of large tumors with an average initial volume of 600 mm^3. Tumor regressions in monotherapy were also achieved when treating subcutaneous xenografts of mesothelioma (NCI-H226), gastric (MKN-28), and triple negative breast (HCC70) cancer cell lines in athymic nude mice. Highly synergistic antitumor efficacy was observed in combination therapy with paclitaxel when treating subcutaneous xenografts of the recombinant high mesothelin expressing A431/H9 cell line or the pancreatic cancer cell line KLM1. Synergy was also observed in the HCC70 and MKN-28 cell lines. These results support evidence that LMB-100 in monotherapy or in combination with standard chemotherapies may provide clinical benefit to patients with cancer.

1.2.3.3.2 Pharmacokinetics in Animals

The pharmacokinetics (PK) of LMB-100 were tested in cynomolgus monkeys following a single IV administration at doses ranging from 0.03 mg/kg to 0.3 mg/kg. Two different enzyme-linked immunosorbent based formats were used for analyzing plasma levels of LMB-100; free and total drug assay (where the total drug assay was the sum of free LMB-100 and LMB-100 complexed with binding molecules). LMB-100 showed a relatively rapid plasma clearance and a volume of distribution at steady-state similar to the plasma volume. Within the dose range tested, non-linear PK was observed for free drug with an extended half-life at higher doses (mean terminal half-life approximately 0.6 hours at 0.3 mg/kg compared to 0.3 hours at 0.03 mg/kg) suggesting saturation of MSLN-mediated clearance pathways. Clearance of total drug was consistently lower than that for free drug implying the presence of soluble binding partners such as soluble mesothelin and ADAs. Induction of anti-drug antibodies (ADA) responses was frequently detectable in all dose groups tested. Overall, given the limited predictive value of immunogenicity reactions in animals to human, a risk for immunogenicity in humans cannot be excluded. Toxico-kinetics after repeated IV dosing in cynomolgus monkeys demonstrated an increase in total exposure in a dose proportional manner between 0.1 mg/kg and 3.0 mg/kg. No accumulation was observed over 5 consecutive days of treatment or over two dosing cycles with 3 × per week dosing. Almost all

monkeys developed ADAs upon treatment, while induction of high ADA levels impaired the exposure of free drug. In some cases the induction of ADAs may have induced a slight increase in exposure

The relationship between systemic drug exposure and anti-tumor activity of LMB-100 was investigated on human lung cancer NCI-H596 xenograft growth in female SCID beige mice. Free and total drug profiles were similar in mice. Modeling estimated a plasma concentration of 6800 ng/mL (± 36%) to trigger a half maximal rate of tumor regression. Concentrations of LMB-100 above this level resulted in potent tumor regression after dosing. Normalized for exposure, SS1P was found to be ~3-fold more potent than LMB-100 in terms of tumor growth inhibition.

1.2.3.3.3 Toxicology and Safety Pharmacology

The toxicological profile of LMB-100 was assessed after repeated intravenous administration to cynomolgus monkeys, the only relevant species, for a maximum of 5 daily doses for one week or 2 cycles with QOD × 3 dosing, separated by a 9 day dosing free period. Four daily doses of 3 mg/kg exceeded the maximum tolerated dose with animals being found in moribund condition, indicated by clinical signs of hypoactivity, hunched posture, ataxia, and tremors. There were no histopathological changes to account specifically for the deteriorating physical condition of these animals.

Histopathological findings such as kidney tubular degeneration/regeneration and changes at serosal-lining tissues were observed at lower doses as well. In general, administration of LMB-100 resulted in both on- and off-target toxicities.

On-target effects were observed on serosal-lining tissues, consistent with high expression of mesothelin. Mesothelium hypertrophy accompanied by subpleural cellular hypertrophy and serosal fibrin exudate was observed in the lung at doses ≥ 1 mg/kg. Mesothelium hypertrophy also occurred in heart (epicardium), spleen, and stomach. Off-target or non-specific toxicity included degeneration/regeneration of kidney tubular epithelium after repeated doses of ≥ 0.3 mg/kg. Local inflammatory findings at the injection sites were observed after administration of LMB-100 in several studies. Clinically, reddening of the skin, swelling, and skin being warm to touch or flaky injection sites were reported. In the 2-cycle GLP study (3 intermittent doses over a 5-day period, 9-days apart), impaired movement of animals from all dose groups was likely related to injection site findings and an overall inflammatory profile. One female at 1 mg/kg was sacrificed early on Day 4 after 2 doses due to severe clinical signs most likely attributed to inflammatory changes at injection sites resulting in moribundity of the animal. Clinically observed inflammatory changes correlated with histopathological changes such as hemorrhages and/or acute inflammation at the injection sites and clinical pathology changes consistent with an overall inflammatory profile (increases in monocytes, neutrophils, CRP, and haptoglobin). Microscopic changes reversed completely after the 4-week recovery period in the 2-cycle GLP study. The Highest Non Severely Toxic Dose in this study was 0.3 mg/kg, which resulted in a mean AUC for total drug of 16.0 µg·h/mL (study day 1, preliminary data). In a subsequent 1 cycle GLP study (QOD × 3 dosing), markedly reduced Injection site findings were observed after administration of a batch with reduced levels of product related modifications of LMB-100. In this study, the HNSTD was 1 mg/kg, resulting in an AUC for total drug of 27.4 and 23.6 µg·h/mL after the first and third dose (preliminary data).

The potential of LMB-100 to induce off-target vascular leak in lungs was assessed in female Wistar rats. Mild perivascular edema was reported microscopically, but did not correlate with

macroscopic or serum chemistry findings consistent with VLS. Ultrasound evaluation in the NHP GLP study revealed minimal accumulation of pericardial fluid with limited biological significance at the highest dose of 1 mg/kg. No appreciable accumulation of pleural fluid was observed at necropsy.

In vitro evaluation of LMB-100 in human whole blood assay indicated a low risk for cytokine-mediated infusion related reaction (IRR)/cytokine release syndrome (CRS) upon first administrations. LMB-100 caused no hemolysis when added to human peripheral blood up to the highest concentrations of 0.5 mg/L.

1.2.3.3.4 LMB-100 (formerly RG7787) plus *nab*-paclitaxel (Abraxane) against mesothelioma patient derived xenografts (PDX)

Since our prior studies have shown remarkable synergy between immunotoxins and taxanes we evaluated the efficacy of *nab*-paclitaxel with LMB-100. We chose to use *nab*-paclitaxel instead of paclitaxel since clinical trials of paclitaxel have shown no activity in patients with mesothelioma.[25, 26] *Nab*-paclitaxel is an albumin bound paclitaxel that has distinct pharmacologic properties compared to paclitaxel including greater uptake by and retention within tumor, which could make it efficacious to treat solid tumors.[27] Potential toxicities of *nab*-paclitaxel are listed in section 14.2.2 and product package insert.

Primary mesothelioma cells are much more sensitive to LMB-100 than SS1P:

Primary mesothelioma cell lines NCI-Meso16, NCI-Meso19, NCI-Meso21 and NCI-Meso29 established from patients were evaluated for sensitivity to the anti-mesothelin immunotoxins.[28] All four of these cell lines are sensitive to killing by LMB-100 with IC_{50} of 0.3 to 10 ng/ml with NCI-Meso21 being most sensitive with an IC50 of 0.3 ng/mL (Figure 3). Surprisingly, most of these primary mesothelioma cells except NCI-Meso16 were 2 to 9-fold more sensitive to LMB-100 than SS1P. In case of *nab*-paclitaxel, NCI-Meso16, NCI-Meso21 and NCI-Meso29 were sensitive to *nab*-paclitaxel with IC_{50} of 10, 20 and 25 ng/ml respectively, while as NCI-Meso19 was resistant with IC50 >100ng/mL (Figure 3).

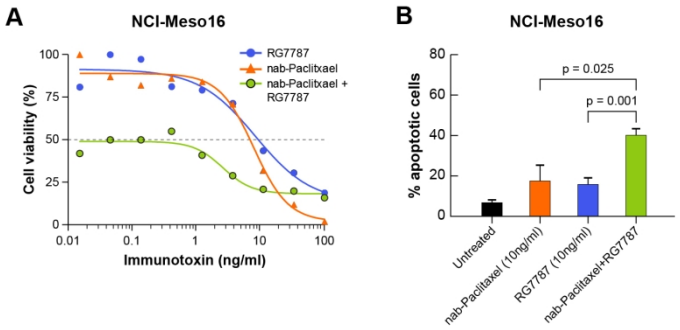
Figure 3: Cytotoxicity of LMB-100 and *nab*-paclitaxel against primary mesothelioma cell lines

Cell lines	Mesothelin sites/cell (10^3)	IC50 (ng/ml)		
		RG7787	SS1P	nab-Paclitaxel
NCI-Meso-16	249	10	1.0	10
NCI-Meso-19	41	1.2	3.7	>100
NCI-Meso-21	346	0.3	2.3	20
NCI-Meso-29	44	1.0	10	25

The *in-vitro* activity of LMB-100 in combination with *nab*-paclitaxel was evaluated using NCI-Meso16, NCI-Meso21 and NCI-Meso29 cell lines since they are sensitive to both LMB-100 and *nab*-paclitaxel. The combination of LMB-100 and *nab*-paclitaxel resulted in decreased cell viability compared to untreated cells, LMB-100 or *nab*-paclitaxel alone treated cells for all three cell lines. This was especially pronounced for NCI-Meso16 cells that had an IC_{50} of 0.01 for LMB-100 plus *nab*-paclitaxel compared to their single agent IC_{50} of 10 ng/ml (Figure 4A). There was

also a sharp increase in the % of apoptotic cells when *nab*-paclitaxel and LMB-100 were used in combination vs. *nab*-paclitaxel or LMB-100 alone (Figure 4B).

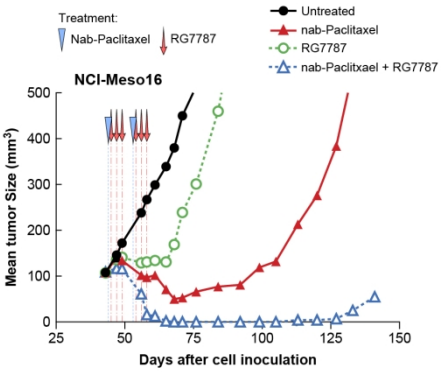
Figure 4: In-vitro synergy between LMB-100 and *nab*-paclitaxel (A) as well as induction of apoptosis (B) against NCI-Meso16 cells.



LMB-100 plus *nab*-paclitaxel have marked anti-tumor efficacy against mesothelioma PDX models:

Figure 5 describes the anti-tumor activity of LMB-100, *nab*-paclitaxel or combination of the two against NCI-Meso16 tumor xenografts. As shown, treatment with LMB-100 or *nab*-paclitaxel slowed tumor growth but after treatment was stopped the tumors rapidly increased in size. However, in mice that were treated with LMB-100 plus nab-paclitaxel there was complete tumor regression in all mice with 5 /7 mice being tumor free when the experiment was terminated on day 142. Evaluation of tumors in mice treated with LMB-100 plus *nab*-paclitaxel showed increased apoptosis, decreased Ki-67 staining and decreased mesothelin positive cells compared to LMB-100 or *nab*-paclitaxel alone treated mice. Similar anti-tumor efficacy of LMB-100 plus *nab*-paclitaxel was also seen against NCI-Meso21 and NCI-Meso29 PDX models. These pre-clinical studies provide strong rationale for clinical trial of LMB-100 alone or with *nab*-paclitaxel to treat patients with mesothelioma.

Figure 5: Anti-tumor efficacy of LMB-100 plus *nab*-paclitaxel against mesothelioma PDX NCI Meso-16.

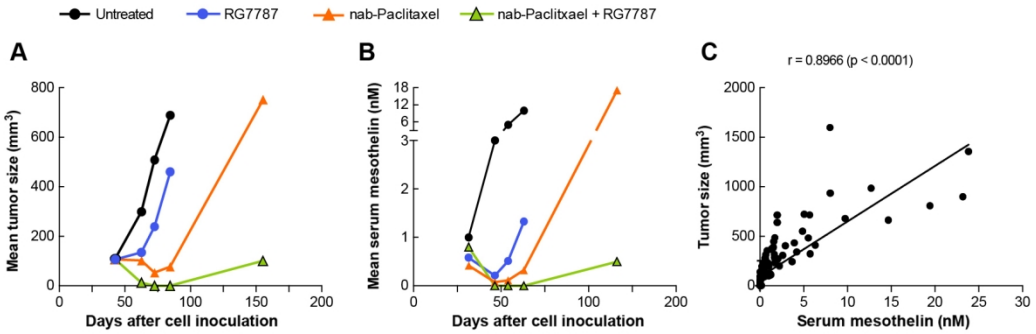


Serum mesothelin is a biomarker of tumor response in mesothelioma PDX models:

Since the NCI-Meso16, NCI-Meso21 and NCI-Meso29 tumors highly express mesothelin we hypothesized that these tumors could release human mesothelin in mouse blood and therefore

measuring the levels could be used to assess response to therapy. As shown in Figure 6 in mice with NCI-Meso16 tumors, the serum mesothelin levels increased in the control group and decreased in the LMB-100 and *nab*-paclitaxel group and became undetectable in the combination group. There was excellent correlation between tumor size and serum mesothelin levels in these mice (r=0.89; p<0.0001). Similarly, in the NCI-Meso21 and NCI-Meso29 tumor model there was very good correlation between tumor size and serum mesothelin in the different treatment groups. These results provide pre-clinical evidence that serum mesothelin is a robust marker of tumor response in mesothelioma and supports earlier clinical observations by us and others that serum mesothelin levels correlate with radiologic tumor response in patients.[29-31]

Figure 6: Correlation between tumor size, tumor response and serum mesothelin for NCI-Meso16 tumors.



Based on these data, and the lack of response to single agent LMB-100 (see section 1.2.3.5), with this amendment two additional arms will be added to the study to receive combination therapy with LMB-100 and *nab*-paclitaxel.

1.2.3.3.4.1 *Nab*-paclitaxel dose justification

The standard dose for *nab*-paclitaxel in non small cell lung cancer is 100 mg/m^2 on days 1, 8 and 15 of each 21 day cycle. The same dose will be used on this study; however, administration will be less frequent (days 1 and 8 of each 21-day cycle) in order to decrease possible toxicity due to the combination. Given the expected side effects of *nab*-paclitaxel including peripheral sensory neuropathy, fatigue and grade 3/4 neutropenia we have reduced the dose frequency of *nab*-paclitaxel by 33% to 100 mg/m^2 given on days 1 and 8 of each 21-day cycle.

1.2.3.4 Clinical testing of single agent LMB-100 (Roche Study)

Initial clinical testing of LMB-100 was performed by Roche in a multi-center international first in human trial (NCT02317419). The primary objective of the Phase I study was to define the safety and tolerability (including the MTD) and pharmacokinetics of the drug in participants with MSLN-expressing metastatic or locally advanced solid tumors for whom no standard therapy was available. Secondary objectives included determination of the RP2D and schedule, exploration of preliminary anti-tumor activity by assessing objective response rate (ORR) and disease control rate (DCR), and assessment of pharmacodynamic effects.

A total of 15 participants were enrolled onto the study before termination. Median age of participants was 60.8 yrs and 53.3% were female. All participants had received prior anti-cancer therapy for their tumors. Enrolled participants had advanced mesothelioma (7), ovarian cancer (3),

pancreatic cancer (3), and gastroesophageal cancer (2). Tumors from 13 of the 15 participants treated had moderately to strongly positive MSLN expression as measured by central IHC analysis.

LMB-100 was administered intravenously on Days 1, 3 and 5 of a 21 day treatment cycle. No pre-medications were given. Treatment was initiated at the MTD of SS1P, 45 mcg/ kg. Five different dose levels were tested (see Table 1). Dose limiting toxicity (DLT) was reached at 250 mcg/kg, with 2 of 4 participants treated at this dose level experiencing vascular leak syndrome (grade 2 and grade 4). Additional toxicities were associated with this dose level. At this point, a sixth cohort receiving 200 mcg/kg of study drug was enrolled, however, the study was terminated by the company before the two accrued participants completed cycle 1 of therapy. Therefore the single agent MTD was not determined.

Table 1. **LMB-100 Dose escalation study- NCT02317419**

Dose (mcg/kg)	No. of patients	Pts with DLT
45	1	0
65	1	0
100	3	0
170	4	0
200	2	NE
250	4	2

DLTs were vascular leak syndrome and proteinuria

NE, Study terminated before DLT assessment period was complete and patients only received single dose of LMB-100

1.2.3.4.1 LMB-100 Adverse Events

Overall, 14 participants (93.3%) experienced at least one AE. The most common AEs were hypoalbuminemia (60.0%), fatigue (53.3%), peripheral edema (53.3%), nausea (46.7%), pyrexia (40.0%), decreased appetite (33.3%), dyspnea (33.3%), and myalgia (33.3%). SAEs included vascular leak syndrome, pyrexia, atrial flutter, infusion related reaction, arthritis, glomerulonephritis minimal lesion and dyspnea. No participants experienced an AE that led to withdrawal of study treatment. Four participants experienced a total of 8 infusion-related reactions that were independent of drug dose level. All of these AEs were non-serious and resolved within approximately 1 hour of onset. Pre-medication for infusion reaction was administered to these participants prior to subsequent doses of LMB-100. Two suspected Type III hypersensitivity reactions were observed. These consisted of arthritis (1 patient) and rash with fever (1 patient), both of which were fully reversible. When other AEs attributed to the study drug are presented by dose level of drug, it becomes clear that toxicity was strongly associated with the 250 mcg/kg dose level at which DLT was reached. Two of four patients treated at 250 mcg/kg experienced serious

VLS which manifested with hypotension, respiratory compromise, serosal membrane reaction and hyponatremia as well as the hypoalbuminemia and edema that can be seen with mild VLS. Other symptoms associated with the DLT dose were fatigue, nausea, vomiting, decreased appetite and mild elevation of transaminases. Table 2 summarizes any adverse event related to LMB-100 while as Table 3 summarizes grade 3 or 4 toxicity related to LMB-100. As shown in Table 3 most grade 3 or 4 toxicity were seen in patients treated at 250 mcg/kg dose level. However, patients receiving LMB-100 doses less than 250 mcg/kg had mainly grade 1-2 adverse events except 1 patient having grade 3 arthritis and 1 patient with grade 3 anemia.

Table 2: Adverse Events attributed to LMB-100[1]

Dose (mcg/kg)	45	65	100	170	250	**ALL**
# of patients treated	1	1	3	4	4	**13**
Vascular Leak	-	-	1	1	2	**4**
grade 3 or 4 vascular leak	-	-	-	-	1	**1**
hypotension	-	-	-	-	2	**2**
hypoalbuminemia	-	1	3	2	3	**9**
peripheral edema	1	1	3	2	4	**11**
facial edema	-	1	2	-	1	**4**
weight gain	-	-	1	-	1	**2**
hyponatremia	-	-	-	-	3	**3**
hypophosphatemia	-	-	-	-	1	**1**
dyspnea	-	1	-	-	2	**3**
Infusion related reaction	-	-	2	-	2	**4**
Constitutional						
fatigue	-	-	-	1	4	**5**
asthenia	-	-	3	-	-	**3**
fever	1	-	2	1	2	**6**
Musculoskeletal						
myalgia	-	-	1	1	2	**4**
arthralgia	1	-	-	-	1	**2**
arthritis	-	-	1	-	-	**1**
muscle spasm	-	-	-	1	-	**1**
Cardiac						
pericardial effusion	-	-	-	-	1	**1**
Atrial flutter	-	-	-	1	-	**1**
Renal Disorders						
glomerulonephritis minimal	-	-	-	1	-	**1**
proteinuria	-	-	-	1	1	**2**
Creatinine increase	-	-	-	-	2	**2**
Gastrointestinal						
decreased appetite	-	1	-	1	2	**4**
nausea	1	-	1	1	4	**7**
abdominal pain	-	1	-	1	1	**3**

Dose (mcg/kg)	45	65	100	170	250	**ALL**
# of patients treated	1	1	3	4	4	**13**
diarrhea	1	-	1	-	-	**2**
vomiting	-	-	-	-	2	**2**
abdominal distension	-	1	-	-	-	**1**
constipation	-	-	-	1	-	**1**
dyspepsia	-	-	-	1	-	**1**
AST increase	-	-	-	-	2	**2**
Hematologic						
anemia	-	-	-	1	-	**1**
decreased lymphocytes	-	-	-	-	1	**1**
Total Grade 3 or greater	-	-	2	1	2	**5**

Orange highlighting indicates that one of the patients experienced a grade 3 or 4 toxicity in this category. Please note that there was only 1 patient who experienced a high grade toxicity of each type.

[1] adapted from Clinical Study Report No 1066017 from Roche dated December 2015, "Summary of Adverse Events Related to Study Medication, Safety-Evaluable Patients Protocol: BP29387" See pages 175-193 of the report."

Table 3: Grade 3 or 4 Adverse Events Attributed to LMB-100

Dose (mcg/kg)	45	65	100	170	250	**ALL**
# of patients treated	1	1	3	4	4	**13**
Vascular leak (gr 4)	-	-	-	-	1	**1**
Hyponatremia (gr 3)	-	-	-	-	1	**1**
Anemia (gr 3)	-	-	-	1	-	**1**
Decreased lymphocytes (gr 3)	-	-	-	-	1	**1**
Dyspnea (gr 3)	-	-	-	-	1	**1**
Infusion-related reaction (gr 3)	-	-	1	-	-	**1**
Arthritis (gr 3)	-	-	1	-	-	**1**

[1] adapted from Clinical Study Report No 1066017 from Roche dated December 2015, "Summary of Adverse Events Related to Study Medication, Safety-Evaluable Patients Protocol: BP29387" See pages 175-193 of the report.

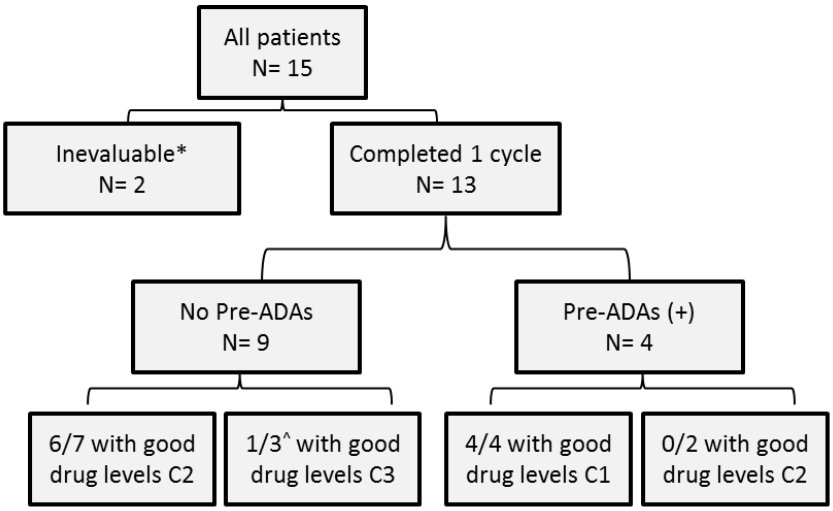
1.2.3.4.2 Anti-Drug Antibodies (ADAs) and LMB-100 Drug Levels

Twelve participants were evaluable for efficacy. The best confirmed overall response was stable disease in 3 participants. A Roche-developed ELISA test was used to retrospectively assess anti-drug antibody (ADA) titers. Four of 13 patients had detectable ADAs at study enrollment while the remaining participants did not; however, the remaining 10 participants developed detectable ADAs by the end of Cycle 2. (Figure 7**,** Table 4 and Table 5). All evaluable participants achieved expected serum drug levels during the first cycle of treatment. Six of 7 participants without pre-existing ADAs achieved effective drug levels during the second cycle, while 0 of 2 participants with pre-existing ADAs did. One of 3 participants that received a third cycle of treatment also achieved effective drug levels during this cycle. A positive test for ADAs did not definitively predict poor blood levels in the subsequent cycle (see patient 1101 in Table 5, who had very good LMB-100 blood levels despite the presence of ADA).

In summary the data regarding LMB-100 ADA and LMB-100 blood levels show:

- Presence of ADA is not predictive of ability to achieve measurable LMB-100 concentration in the serum, which is the most important parameter for drug efficacy.
- Patients who have presence of pre-existing ADA are able to achieve good LMB-100 blood levels during cycle 1 (4/4 patients), but may not do so during cycle 2 (2/2 patients with undetectable blood levels) although the numbers are small.
- Patients who do not have presence of pre-existing ADA are able to achieve good LMB-100 blood levels during cycle 1 (7/7 patients) as well as cycle 2 (6/7 patients).
- Very few patients have been to determine LMB-100 blood levels during cycle 3 and beyond. However, 1/3 patients had good LMB-100 blood levels that were in fact higher than those achieved during cycle 1 and 2 despite the presence of ADA. In addition, another patient had low but detectable blood levels during cycle 3. More patients need to be treated to determine what percent of patients will achieve good blood levels during cycle 3 and beyond.

Figure 7: Effect of anti-drug antibodies (ADA) on the ability to achieve good LMB-100 blood levels in patients treated with LMB-100.



*Roche closed the study before these patients completed their first treatment cycle
^One additional patient has no C3 PK data in the record but is recorded as not having progression until C4D1

Table 4: Effect of ADA on LMB-100 blood levels in patients without pre-existing ADA.

The table shows patients treated at different dose levels of LMB-100; ADA prior to start of each cycle and LMB-100 blood levels as Cmax (ng/ml). In addition, the change in LMB-100 Cmax concentration (as percent increase or percent decrease) during cycle 2 and 3 is shown as dC1/dC2 and dC1/dC3 respectively. Please note that – means the PK assay was not performed.

Patient	Dose (mcg/kg)	Cycle 1		Cycle 2			Cycle 3		
		ADA (Day1)	Cmax (ng/ml)	ADA (Day1)	Cmax (ng/ml)	dC1/dC (%)	ADA (Day1)	Cmax (ng/ml)	dC1/dC (%)
1002	65	0	1150	8100	711	-38	-	-	-
1401*	100	0	1790	0	1610	-10	24300	267	-85
1402	100	0	1650	0	1360	-18	8100	0	-100
1101*	170	0	2760	300	3950	43	900	3490	26
1202	170	0	3040	0	1940	-36	-	-	-
1301	170	0	3430	72900	527	-85	-	-	-
1403	170	0	1930	900	1550	-20	-	-	-
1102*	250	0	5480	0	4770	-13	-	-	-
1302	250	0	4340	-	-	-	-	-	-

* Patient's treatment stopped due to study closure

ADA data are from Roche "Bioanalytical Report (ADA) for Clinical Study BP29387"with ADA time point codes translated as specified in the BP29837 Lab Manual Version 1.0. C_{max} data were taken from the Roche final study report.

Table 5: Effect of ADA on LMB-100 blood levels in patients with pre-existing ADA.

The table shows patients treated at different dose levels of LMB-100; ADA prior to start of each cycle and LMB-100 blood levels as Cmax (ng/ml). In addition, the change in LMB-100 Cmax concentration (as percent increase or percent decrease) during cycle 2 and 3 is shown as dC1/dC2 and dC1/dC3 respectively. Please note that – means the PK assay was not performed.

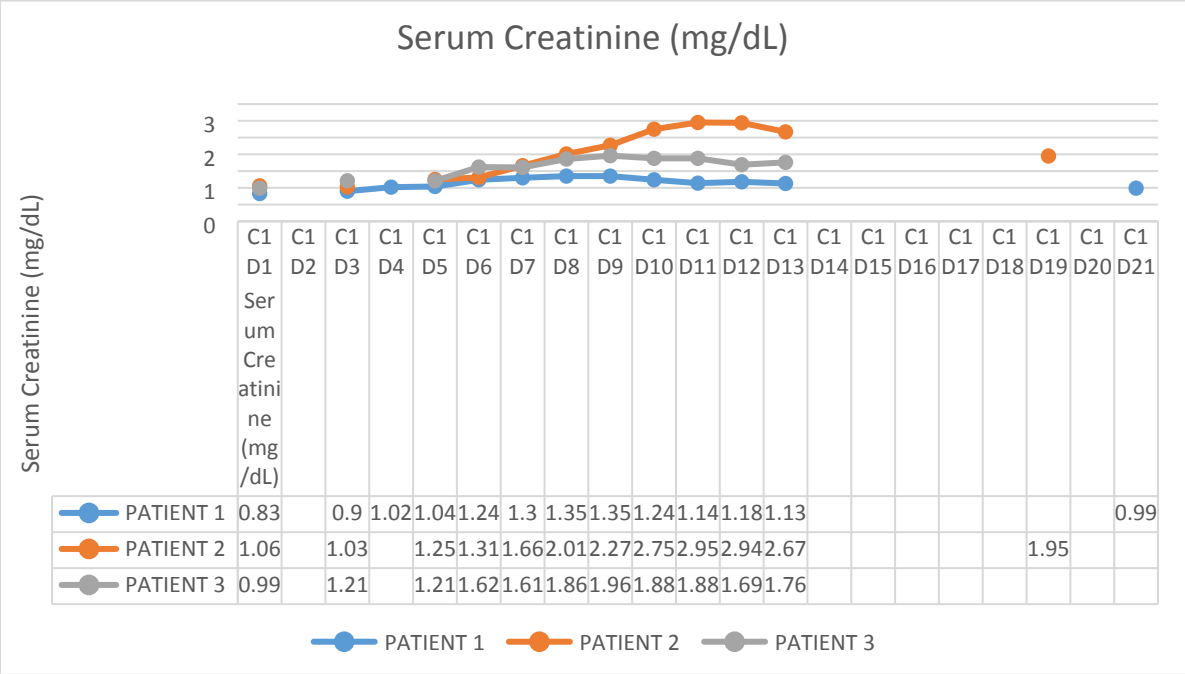
Patient	Dose (mcg/kg)	Cycle 1		Cycle 2		
		ADA (Day1)	Cmax (ng/ml)	ADA (Day1)	Cmax (ng/ml)	dC1/dC2 (%)
1001	45	2700	620	196830	0	-100
1201	100	8100	495	196830	0	-100
1501*	250	2700	3730	196830	-	-
1003	250	900	2960	196830	-	-

* Patient's treatment stopped due to study closure

1.2.3.5 Clinical testing of single agent LMB-100 (Current Study – 16C0127 as of 8/15/17)

1.2.3.5.1 Arms A1 and A2

Figure 8: Serum Creatinine for Patients 1, 2 and 3 treated at 170 µg/kg



As of December 20, 2016 when Arms A1 and A2 (single agent LMB-100) were closed and Arms B1 and B2 (LMB-100 + *nab*-paclitaxel) were established ten patients were enrolled on the study. Out of these 10 patients 7 had peritoneal mesothelioma and 3 pleural mesothelioma; 6 female and 4 male and median age was 62 years old. The first 3 patients were treated at dose level 1 i.e. 170 mcg/kg with patient #1 initiating treatment on July 28, 2016. All three patients at this dose level during cycle 1 had grade 1 or 2 increase in serum creatinine (Figure 8). Since increase in serum creatinine was a common toxicity pattern at this dose level it was defined as DLT as per protocol established criteria (per bullet one under Other Toxicities in section 3.1.1). The protocol was subsequently amended (Amendment B dated 08/23/16) to allow the treating these three patients at dose level -1 (140 mcg/kg) during cycle 2-4 though the protocol prior to the amendment would have allowed retreatment at 170 mcg/kg as long as they met inclusion criteria for the study especially adequate renal function, defined in the protocol as creatinine clearance (by Cockcroft Gault formula) ≥50 mL/min. However, we felt it would be safer to re-treat these patients at dose level-1 instead of dose level 1 and adjusted the dose modification section (3.3.1) of the protocol accordingly as part of Amendment B.

All subsequent patients were treated at 140 mcg/kg. Patients 001-003 have completed 4 cycles of LMB-100 treatment (LMB-100 170 mcg/kg during cycle 1 and LMB-100 140 mcg/kg during cycle 2-4) and seven additional patients have been enrolled at dose level -1 (140 mcg/kg). No DLT's have been observed at the LMB-100 dose level of 140 mcg/kg. Therefore, the recommended phase of single agent LMB-100 is 140 mcg/kg given every 3 weeks.

Grade 2 infusion reactions were seen in 5 patients at some point during the treatment with LMB-100. In 2 patients it was seen during cycle 2 and 3 patients during cycle 3 and cycle 4 of treatment. In most case the infusion reaction was managed by administration of dexamethasone and increasing infusion duration as per protocol. However, in two patients who had infusion reaction during cycle 4 of LMB-100 the treatment was discontinued.

No serious adverse events were observed. The most frequently occurring events were hypoalbuminemia (11 events), anemia (6), increased creatinine (4), decreased lymphocytes (4), hyperglycemia (3), dyspepsia (2) and fever (2). There were single occurrences of limb edema, decreased platelets, weight gain and weight loss. Most of the events were grade 2 with the exception of one grade 3 decreased lymphocytes and three grade 3 hyperglycemia.

Out of 10 patients treated with LMB-100 alone who have been evaluated for tumor response after cycle 2, 9 had stable disease and 1 had progressive disease.

1.2.3.5.2 Arm B1

As of August 15, 2017, 6 patients have been treated at Arm B1's dose level 1 (i.e. LMB-100: 100 mcg/kg on days 1, 3 and 5 plus *nab*-paclitaxel: 100 mg/m^2 on days 1 and 8 of a 21-day cycle. Of these 6 patients, 1 patient was not evaluable for toxicity since he did not receive day 8 *nab*-paclitaxel during cycle 1. Of the 5 patients who were evaluable for toxicity we had one DLT (grade 4 myositis/CPK elevation).

1.2.3.5.3 Pharmacokinetics of LMB-100:

Free LMB-100 plasma concentrations were measured with a validated ELISA with a lower limit of quantification of 2.1 ng/mL. Doses ranged from 140 mcg/kg – 170 mcg/kg. Samples for pharmacokinetic (PK) analysis were obtained from patients at pre-dose, end of infusion (EOI; 30-min post start), and 1 hour, 2 hours, 3 hours, 4 hours and 6 hours post EOI. Concentration data for each dose was plotted over time to assess the impact of increasing anti-drug antibodies (ADAs) that are generated in response to LMB-100 exposure. PK analysis was done for the first five patients treated on the study and is shown in Table 6 below. Measured LMB-100 plasma concentrations were consistent during the first week of treatment, with a near dose-proportional increase in C_{MAX} from 140 mcg/kg to 170 mcg/kg. However, the suspected generation of ADAs greatly reduced LMB-100 exposure by cycle 2.

As shown in Table 6, all 10 patients had good LMB-100 blood levels during cycle 1. However only 5 of 10 patients had detectable blood levels during cycle 2. None of the 9 patients who got cycle 3 of LMB-100 had good LMB-100 blood levels during cycle 3; and none of 6 patients had good LMB-100 blood levels during cycle 4.

Table 6: Decrease in LMB-100 Exposure Over Time

Patient	C1D1		C2D1		C3D1		C4D1	
	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)
1	170	1991	140	1091	140	21	140	21

Patient	C1D1		C2D1		C3D1		C4D1	
	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)
2	170	2286	140	297	140	70	140	21
3	170	2760	140	4.4	140	BQL	140	BQL
4	140	1054	140	681	140	BQL	N/A	N/A
5	140	1124	140	BQL	N/A	N/A	N/A	N/A
6	140	2584	140	1099	140	BQL	140	BQL
7	140	3118	140	2450	140	BQL	140	BQL
8	140	1721	140	BQL	140	BQL	140	BQL
9	140	2584	140	1099	140	BQL	140	BQL
10	140	3118	140	2450	140	BQL	140	BQL

***** BQL: below assay's quantifiable limit (2.1 ng/mL); N/A: data not available

Note: Anti-drug (LMB-100) antibodies (ADAs) were measured pre-dose on C1D1. Inhibition percentages ≥41.8% indicate presence of pre-existing ADAs

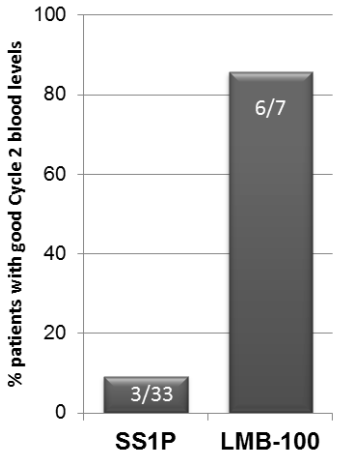
These results are in agreement with the Roche phase I clinical trial. It is also clear that administration of LMB-100 beyond cycle 2 is unlikely to result in meaningful clinical benefit since there are no detectable blood levels during cycle 3 and 4. Based on the result of the data from the first 5 patients, as of Amendment C (dated 03/09/17) LMB-100 will only be administered during cycles 1 and 2 in patients enrolled subsequent this amendment (Arms B1 and B2). Previously enrolled subjects will continue to receive 4 cycles of LMB-100 as PK data are not yet complete.

1.2.4 Comparison of toxicity and immunogenicity of LMB-100 to SS1P

LMB-100 was safely administered to four patients at 170 mcg/kg without DLT. This is nearly four times the maximum tolerated dose (MTD) of SS1P. Most patients without pre-existing ADAs were able to achieve effective drug levels of LMB-100 for 2 cycles, unlike what has been seen previously with SS1P (see Figure 2 and Figure 9). These data demonstrate that LMB-100 is both less toxic and less immunogenic than SS1P.

Figure 9: The ability to achieve good drug levels during cycle 2 is shown for patients who were treated on a phase I trial of single agent SS1P[12] and those treated with LMB-100.

Only 9% of patients treated with SS1P had good blood levels during cycle 2 versus 86% for patients treated with LMB-100 ($p>0.001$ by two tailed Fisher's exact test).



1.2.5 Rationale for the study

There is clearly an unmet need to identify improved therapies for patients with malignant mesothelioma. LMB-100 is a reduced immunogenicity anti-mesothelin RIT developed in NCI's Laboratory of Molecular Biology in collaboration with Roche. Mesothelin is expressed in over 95% of epithelioid subtype of malignant mesothelioma. Limited dose escalation completed to date indicates safety and provides preliminary data on immunogenicity. Identification of the recommended phase 2 dose (RP2D), now established at 140 mcg/kg and preliminary assessment of its monotherapy efficacy will inform future studies of LMB-100 in combination with other agents.

As of Amendment C, we also began to evaluate the efficacy and tolerability of 2 cycles of LMB-100 at the established RP2D in combination with *nab*-paclitaxel (Arm B) in order to explore the synergy between the agents as addressed in section 1.2.3.3.4. Initially, we planned to study 2 escalating dose levels of LMB-100, 100 mcg/kg (dose level 1) and 140 mcg/kg (dose level 2) with a stable dose of *nab*-paclitaxel (100 mg/m^2). However, as of Amendment F, it was determined that that the highest dose level tested would be dose level 1 based on data from a companion protocol (16C0128) in which LMB-100+*nab*-paclitaxel is used the treatment of patients with pancreatic cancer. In this study, 2 of 6 subjects treated with an LMB-100 dose of 100 mcg/kg and a *nab*-paclitaxel dose 125 mg/ m^2 experienced DLT. Therefore, we have decided not to evaluate dose level 2. Dose de-escalation may occur if more than 1 DLT occurs at the initial dose level,

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria (All Arms)

2.1.1.1 Histologically confirmed epithelial or biphasic mesothelioma not amenable to potentially curative surgical resection. However, patients with biphasic tumors that have a ≥ 50% sarcomatoid component will be excluded. The diagnosis will be confirmed by the Laboratory of Pathology, CCR, NCI.

2.1.1.2 Archival sample or fresh biopsy or tumor effusion must be available for confirmation of diagnosis.

2.1.1.3 Patients must have measurable disease per RECIST 1.1. as defined per . (See Section 6.3).

2.1.1.4 Patients must have had at least one prior chemotherapy regimen that includes pemetrexed and cisplatin or carboplatin. There is no limit to the number of prior chemotherapy regimens received.

2.1.1.5 The last dose of previous therapy must have occurred at least 3 weeks prior to the start of study therapy. Palliative radiotherapy is allowed up to 2 weeks before the first LMB-100 infusion.

2.1.1.6 Patients for whom no standard curable therapy exists

2.1.1.7 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of LMB-100 alone or in combination with *nab*-paclitaxel in patients <18 years of age, children are excluded from this study

2.1.1.8 All acute toxic effects of any prior radiotherapy, chemotherapy, or surgical procedure must have resolved to Grade ≤ 1, except alopecia (any grade) and < Grade 2 peripheral neuropathy.

2.1.1.9 ECOG performance status (PS) 0 or1 (Appendix A)

2.1.1.10 Adequate hematological function: neutrophil count of ≥ 1.5×10^9 cells/L, platelet count of ≥ 100,000/μL (transfusion independent, defined as not receiving platelet transfusions within 7 days prior to laboratory sample), hemoglobin ≥ 9 g/dL

2.1.1.11 Adequate Liver function: AST and ALT < 2.5 X upper limit of normal, alkaline phosphatase < 2.5 X upper limit of normal, unless bone metastasis is present (<5 X upper limit of normal) in the absence of liver metastasis.

2.1.1.12 Bilirubin ≤ 1.5 mg/dL (excluding Gilbert's Syndrome, see below).

2.1.1.13 Patients with Gilbert's syndrome will be eligible for the study. The diagnosis of Gilbert's syndrome is suspected in people who have persistent, slightly elevated levels of unconjugated bilirubin without any other apparent cause. A diagnosis of Gilbert's syndrome will be based on the exclusion of other diseases based on the following criteria:

- Unconjugated hyperbilirubinemia noted on several occasions
- No evidence of hemolysis (normal hemoglobin, reticulocyte count, and LDH)
- Normal liver function tests

- Absence of other diseases associated with unconjugated hyperbilirubinemia

2.1.1.14 Adequate renal function: creatinine < 1.5 mg/dL OR creatinine clearance (by Cockcroft Gault formula Appendix B) ≥ 50 mL/min.

2.1.1.15 Must have serum albumin > 2.5 mg/dL without intravenous supplementation

2.1.1.16 Must have left ventricular ejection fraction > 50%

2.1.1.17 Must have an ambulatory oxygen saturation of > 90% on room air

2.1.1.18 The effects of LMB-100 alone or in combination with *nab*-paclitaxel on the developing human fetus are unknown. For this reason:

2.1.1.18.1 Women of child-bearing potential (defined as a sexually mature woman who (1) has not undergone hysterectomy [the surgical removal of the uterus] or bilateral oophorectomy [the surgical removal of both ovaries] or (2) has not been naturally postmenopausal for at least 24 consecutive months [i.e., has had menses at any time during the preceding 24 consecutive months]) must:

- Either commit to true abstinence* from heterosexual contact (which must be reviewed on a monthly basis), or agree to use, and be able to comply with, effective contraception without interruption, 28 days prior to starting study therapy (including dose interruptions), while on study medication and for 3 months after the last dose of study therapy; and
- Have a negative serum pregnancy test (β -hCG) result at screening and agree to ongoing pregnancy testing during the course of the study, and after the end of study therapy. This applies even if the subject practices true abstinence* from heterosexual contact.

2.1.1.18.2 Men must agree to practice true abstinence* or agree to use a condom during sexual contact with a pregnant female or a female of childbearing potential while participating in the study, during dose interruptions and for 6 months following discontinuation of study therapy, even if he has undergone a successful vasectomy.

2.1.1.19 Ability of subject to understand and the willingness to sign a written informed consent document.

* True abstinence is acceptable when this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception].

2.1.2 Exclusion Criteria (All Arms)

2.1.2.1 Known or clinically suspected CNS primary tumors or metastases including leptomeningeal metastases. History or clinical evidence of CNS metastases unless they have been previously treated, are asymptomatic, and have had no requirement for steroids or enzyme-inducing anticonvulsants in the last 14 days.

2.1.2.2 Evidence of significant, uncontrolled concomitant diseases which could affect compliance with the protocol or interpretation of results, including significant pulmonary disease other than primary cancer, uncontrolled diabetes mellitus, and/or significant cardiovascular disease (such as New York Heart Association Class III or IV cardiac disease, myocardial

infarction within the last 6 months, unstable arrhythmias, unstable angina, or clinically significant pericardial effusion)

2.1.2.3 Active or uncontrolled infections.

2.1.2.4 HIV or active HBV or HCV infection. HIV positive patients will be excluded due to a theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.

2.1.2.5 Patients with prior pneumonectomy

2.1.2.6 Any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that would contraindicate the use of an investigational drug

2.1.2.7 Major surgery or significant traumatic injury ≤ 28 days prior to the first LMB-100 infusion (excluding biopsies) or anticipation of the need for major surgery during study treatment

2.1.2.8 Dementia or altered mental status that would prohibit informed consent

2.1.2.9 Live attenuated vaccinations 14 days prior to treatment

2.1.2.10 Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with LMB-100, breastfeeding should be discontinued if the mother is treated with LMB-100. These potential risks may also apply to other agents used in this study.

2.1.2.11 Known hypersensitivity to any of the components of LMB-100

2.1.2.12 High doses of systemic corticosteroids within 7 days prior to first dosing. High dose is considered as > 20 mg of dexamethasone a day (or equivalent) for > 7 consecutive days.

2.1.3 Exclusion Criteria (Arm B only)

2.1.3.1 Subjects must not have received paclitaxel or *nab*-paclitaxel within 4 months prior to initiation of study therapy

2.1.3.2 Participants with contra-indication and/or history of severe hypersensitivity reactions to *nab*-paclitaxel

2.2 SCREENING EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols)

Screening assessments will be performed within 28 days prior to study enrollment.

- Archival tumor sample for NCI LP confirmation of diagnosis. A block of primary tissue (or 5-10 unstained sections on charged slides) from the time of diagnosis will be required from each patient. Tissue blocks from a known recurrence will be accepted if original tumor samples are unavailable. Referring institutions will send the tumor block or 5-10 unstained sections on charged slides to CCR/NCI for correlative studies and confirmation of diagnosis. A fresh biopsy or tumor effusion sample may be collected if archival tumor tissue is not available.
- History and physical exam
- Vital signs including pulse oximetry

- ECOG performance status
- Urine or serum hCG in women of childbearing potential
- ECG
- Echocardiogram
- Chest X-ray
- CT scan of chest, abdomen and/or pelvis and areas of known or suspected disease involvement; MRI may also be performed when appropriate
- FDG-PET scan
- CBC with differential, Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), creatine kinase, C-reactive protein, Coagulation (PT, PTT, fibrin degradation products)
- Urinalysis
- Viral markers – HBSAg, anti-HCV, anti-HIV

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.3.1 Treatment Assignment Procedures

Cohorts

Letter	**Name**	**Description**
A1	Mesothelioma dose de-escalation	Up to 18 Subjects with epithelial or biphasic mesothelioma enrolled to determine RP2D of LMB-100
A2	Mesothelioma dose expansion	7- 10 (depending on interim efficacy analysis) additional subjects with epithelial or biphasic mesothelioma treated at RP2D of LMB-100
B1	Mesothelioma with no recent nab-paclitaxel dose de-escalation	Up to 12 subjects with epithelial or biphasic mesothelioma who have not been treated with nab-paclitaxel in the past 4 months enrolled to determine the RP2D of LMB-100 +nab-paclitaxel

B2	Mesothelioma with no recent nab-paclitaxel dose expansion	7- 10 (depending on interim efficacy analysis) additional subjects with epithelial or biphasic mesothelioma who have not been treated with nab-paclitaxel in the past 4 months treated at RP2D of LMB-100+nab-paclitaxel

Arms

Letter	Name	Description
A1	LMB-100 dose escalation	De-escalating doses of LMB-100 in up to 18 subjects
A2	LMB-100 dose expansion	Fixed dose of LMB-100 as determined in Arm A1 in up to 16 subjects
B1	LMB-100+ nab-paclitaxel dose escalation	De-escalating doses of LMB-100 + fixed dose of nab-paclitaxel in up to 12 subjects
B2	LMB-100+ nab-paclitaxel dose expansion	Fixed dose of LMB-100 as determined in Arm B1 + fixed dose of nab-paclitaxel in up to 16 subjects

Arm Assignment

Subjects in cohort A1 are assigned to arm A1. Subjects in cohort A2 are directly assigned to arm A2. (Note: Part A of the study is now closed).

Subjects in cohort B1 are assigned to arm B1. Subjects in cohort B2 are directly assigned to arm B2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open-label, single center Phase I study of LMB-100 in patients with advanced mesothelioma. Four arms will be assessed: Arms A1 and A2: LMB-100 monotherapy and Arms B1 and B2: LMB-100 in combination with *nab*-paclitaxel.

- Arms A1 and A2 (closed as of Amendment C- 03/09/17)

Up to 18 patients will be enrolled to test up to 3 de-escalating doses of LMB-100 using a standard 3+3 design (Arm A1). Initially, it was planned that up to 16 patients would be evaluated at the RP2D, now determined as 140 mcg/kg in the dose expansion portion of the study using a two-stage Minimax design (Arm A2). Patients treated at the RP2D during the dose de-escalation portion of the study were included in the count of 16 patients. However, as of amendment C, 10 subjects have been treated at the RP2D and as noted in section 8, enrollment will be temporarily halted after the enrollment of 10 evaluable subjects in order to assess PK and ADA data. As no subjects have demonstrated a partial or complete response at this dose, Arms A1 and A2 will no longer be enrolling. All future subjects will be enrolled to Arms B1 and B2.

Subjects previously enrolled on Arms A1 and A2 will continue to be treated as originally planned, receiving LMB-100 intravenously on days 1, 3 and 5 of each 21 day cycle. A maximum of 4 cycles of treatment will be administered.

- Arms B1 and B2

Up to 12 evaluable patients will be enrolled to test up to 2 de-escalating doses of LMB-100 with a constant dose of *nab*-paclitaxel (Arm B1). Up to 16 patients will be evaluated at the recommended phase 2 dose (RP2D) in the dose expansion portion of the study using a two-stage Minimax design (Arm B2). Patients treated at the RP2D during the dose de-escalation portion of the study will be included in the count of 16 patients.

In both portions of Arm B, patients will receive LMB-100 intravenously on days 1, 3 and 5 of each 21 day cycle for up to 2 cycles and *nab*-paclitaxel on days 1 and day 8 of each 21 day cycle for up to 6 cycles.

- All Arms

Pharmacokinetic measurements will be performed with each cycle. Assessment of anti-LMB-100 antibodies will be performed at the start of each cycle. Response to therapy will be assessed at baseline and then following every two cycles by CT scan. Response will be assessed by RECIST 1.1 or modified RECIST for MPM. Tumor mesothelin expression will be assessed retrospectively. Optional tumor biopsy will be performed in consenting patients when deemed feasible at the following time points: before initiation of therapy (archival tissue may also be used) and after two cycles of therapy.

3.1.1 Dose Limiting Toxicity (Dose de-escalation cohorts only)

For the purpose of this study, a DLT will be defined as any of the following events attributed to LMB-100 (i.e. related to LMB-100) and occurring within 21 days after the first dose of LMB-100, the DLT period:

Hematological toxicities:

- Grade 4 neutropenia (i.e. absolute neutrophil count (ANC) <0.5 x 10^9 cells/L) for a minimum duration of 7 days)
- Grade 3 and 4 febrile neutropenia (i.e. ANC < 1.0 x10^9 cells/L with a single temperature of >38.3°C or a sustained temperature of ≥38°C for more than one hour
- Grade 4 thrombocytopenia (≤25.0 x10^9 cells/L)
- Grade 3 thrombocytopenia associated with bleeding episodes

Grade ≥ 3 non-hematological toxicity with the exception of:

- Alopecia (any grade)
- Grade 3 nausea and vomiting without appropriate treatment
- Grade 3 diarrhea lasting for ≤ 2 days with no fever or dehydration
- Infusion-related reactions **up to and including Grade 3**. They are not considered to be DLTs since, based on experience with monoclonal antibodies, IRRs are idiosyncratic and not dose-related events. Precautions will be taken if IRRs Grade ≥ 2 occur (see Section 3.3.2).
- Asymptomatic ≥ Grade 3 lymphopenia, leukopenia, hypoalbuminemia, dyselectroletemia resolving within 24 hours, increase in alkaline phosphatase.

- **Isolated** Grade 3 fever **(without signs and/or symptoms of an infection)** occurring within 48 hours of LMB-100 infusion and resolving within 48 hours to ≤ Grade 2 and fully resolved within 1 week

Grade ≥ 4 non-hematological toxicity:

- Infusion-related reactions

Other toxicities:

- Any other drug related toxicity considered significant enough to be qualified as a DLT in the opinion of the principal investigator.
- Inability to start cycle 2 within 3 weeks of completing cycle 1 due to drug-related adverse events.

If a patient experiences a DLT of any grade, LMB-100 will be discontinued in the patient; however, *nab*-paclitaxel may be continued per protocol.

3.1.2 Dose De-Escalation (Arm A1)

Six patients will initially be entered at dose level 1. If fewer than 2 patients experience DLT during within 21 days after the first dose of LMB-100 at the initial level, this will be the RP2D. Otherwise, dose de-escalation will proceed in cohorts of 3–6 patients. The RP2D is the dose level at which no more than 1 of up to 6 patients experience DLT during cycle 1. If a patient did not experience DLT and did not finish cycle 1, he or she will not be evaluable for toxicity and will be replaced in the dose level to ensure that at least 3 patients in each cohort have been assessed for the full DLT period prior to moving to the next dose level. If 2 or more DLTs occur at dose level -2, an amendment will be submitted in order to explore dose levels lower than 100 mcg/kg.

Table 7: Arm A1 Dose de-escalation table.

Dose De-escalation Schedule	
Dose Level	**Dose of LMB-100 in µg/kg**
Level 1	170
Level -1	140
Level -2	100

Table 8: Arm A1 Dose de-escalation will follow the rules outlined below.

Number of Patients with DLT at Dose Level 1	**De-escalation Decision Rule**
0 or 1 out of 6	This will be the recommended phase 2 dose

Number of Patients with DLT at Dose Level 1	**De-escalation Decision Rule**
≥ 2 out of 6	Enter 3 patients at the next lower dose level
Number of Patients with DLT at Any Other Given Dose Level	**De-escalation Decision Rule**
0 or 1 out of 3	Enter up to 3 more patients at this dose level. • If fewer than 2 out of the 6 patients evaluated at this dose level experience DLT, this is the recommended phase 2 dose. • If 2 or more out of the 6 patients evaluated at this dose level experience DLT, enter 3 patients at the next lower dose level.
≥ 2 out of 3	Enter 3 patients at the next lower dose level.

3.1.3 ,Dose De-Escalation (Arm B1)

Dose de-escalation will proceed in cohorts of 6 patients. Before proceeding to the next dose level, the last patient enrolled at the previous level must have completed at least one cycle of study therapy. The RP2D is the dose level at which no more than 1 of up to 6 patients experience DLT during cycle 1, and the dose below that at which at least 2 (of ≤6) patients experience DLT. If a patient did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose. If 2 or more DLTs occur at dose level -1, an amendment will be submitted in order to explore LMB-100 dose levels lower than 65 mcg/kg.

Table 9: Arm B1 Dose de-escalation table.

Dose De-escalation Schedule		
Dose Level	**Dose of LMB-100 in µg/kg**	***Nab*-paclitaxel in mg/m^2**
Level -1	65	100
Level 1	100	100

Table 10: Arm B1 dose de-escalation will follow the rules outlined below.

Number of Patients with DLT at a Given Dose Level	**De-Escalation Decision Rule**
≤1 out of 6	This is the recommended phase 2 dose.
≥ 2	Enter up to 6 patients at the next lower dose.

3.2 DRUG ADMINISTRATION

3.2.1 LMB-100

The qualified health care professional responsible for dispensing the study drug will prepare the correct dose according to the cohort allocation of each patient.

LMB-100 will be given as an IV solution on Days 1, 3, and 5 (QOD× 3) of every 21 day cycle (up to 4 cycles for Arms A1 and A2, up to 2 cycles for Arms B1 and B2).

LMB-100 must be administered in a hospital or clinic equipped for IV chemotherapy. Full emergency resuscitation facilities should be immediately available and patients should be under close supervision of the investigator or delegate at all times.

The compatibility and stability of the active ingredient was tested under simulated preparation/administration conditions.

3.2.1.1 General Instructions

1. LMB-100 drug product should be inspected visually for particulates prior to administration.
2. Do not use the solution if there is particulate matter or if it is discolored.
3. Do not shake or freeze the vial contents.
4. Ensure the drug vial content is protected from light during preparation and administration (ambient light conditions are acceptable but avoid exposure to direct sunlight).
5. LMB-100 drug product does not contain any preservatives. Vials are for single use only and partially used vials must not be reused.
6. Any unused product should be kept for drug reconciliation.
7. No dilution of LMB-100 drug product into 0.9% saline bags should be performed.
8. Do not administer as IV push or bolus.
9. Other drugs that require parenteral co-administration (if applicable) should be delivered via separate infusion lines and at separate infusion sites and should not be mixed with the study drug.

3.2.1.2 Specific Instructions

LMB-100 is diluted with 0.9% NaCl (1:10) **in-line** immediately prior to administration (see Figure 10 below).

The undiluted LMB-100 drug product, filled in a disposable syringe, is administered by intravenous syringe infusion using a syringe driver pump.

In order to allow **in-line dilution (1:10) immediately prior** to administration of neat drug product, a side flow with 0.9% NaCl must be applied (as illustrated in Figure 10). An IV infusion pump and syringe driver should be used to control the infusion rate of isotonic 0.9% NaCl solution and LMB-100 respectively.

LMB-100 is administered using peripheral vein access and should not be administered using a central venous catheter.

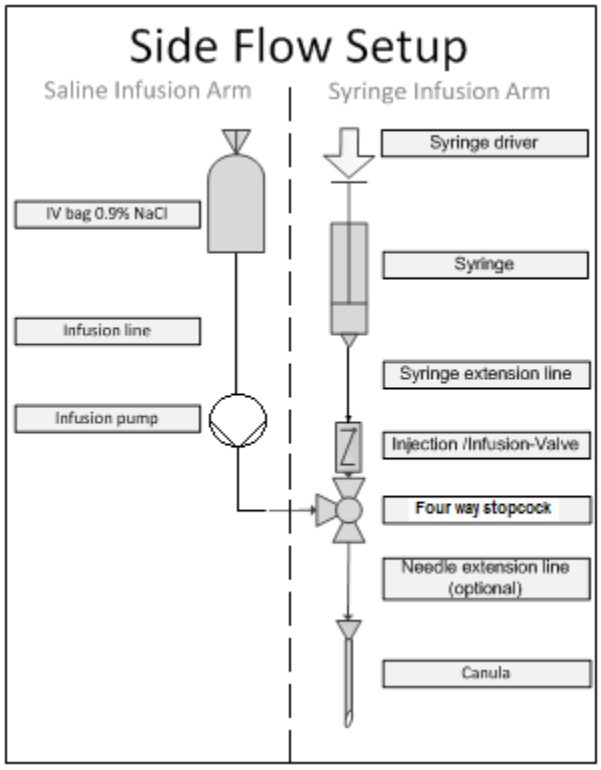


Figure 10: Schematic view of the administration set-up.

The LMB-100 drug product should be filtered during withdrawal from the vial using a BD filter needle or alternatively, an in-line filter may be used as depicted.

1. The syringes for administration must be prepared under appropriate aseptic conditions as LMB-100 drug product does not contain antimicrobial preservatives. All preparation should be conducted under laminar flow hood with safety glasses, lab coat and arm protection and a work procedure to minimize particle contamination.

2. As a measure of precaution, a BD 18G (USA: 19G) blunt filter needle Versapore should be used, if no in-line filter is applied, for the withdrawal of the undiluted drug product (LMB-100) from the vial into the syringe.
3. **Alternatively**, an in-line filter may be used to filter the undiluted drug product (LMB-100) during syringe infusion before side-flow dilution. For this, an in-line filter (0.2 µm pore size) should be positioned between the syringe extension line and the injection/infusion valve (see Figure 10). The filtration must occur **before** in-line dilution with 0.9% saline.
4. Withdraw up to 2 mL excess volume of LMB-100 to the intended dosing volume into the syringe for priming and purging the syringe extension lines before administration (see step 8).
5. The prepared syringe should be stored and transported with a closing cone/stopper.
6. Since LMB-100 drug product does not contain antimicrobial preservatives and to comply with the chemical and physical in-use stability, the prepared syringes for infusion should be used immediately. If not used immediately, total in-use storage times of prepared syringes should not exceed 24 hours to limit microbial growth in case of potential accidental contamination. Storage conditions should generally be at 2°C to 8°C, but syringes may be held at room temperature for up to a maximum of 4 hours. The temperature during syringe preparation, storage and drug administration must not exceed 25 °C.
7. Establish the saline flow first by flushing the lines including the 4-way stopcock and extension lines with saline.
8. Put the filled syringe in the pre-programmed syringe driver, remove the cap and attach the infusion set. Follow the syringe driver manual/local instructions regarding how to set up and prime/purge the system before the line is attached to the closed 4-way stopcock before starting the administration.
9. The end of infusion is defined as the time point at which the syringe driver finishes administering the total volume of LMB-100 to be infused.
10. At the end of the first infusion, the IV line should remain in place for 2 hours. If no infusion related symptoms occur during this time, the infusion line may be removed. For subsequent infusions and if no IRR has been reported, the IV line should remain in place for at least 30 minutes from the end of the infusion. If no adverse events occur during the 30 minutes, the infusion line may be removed. If feasible, the line for drawing blood for PK samples (opposite extremity to the one with the infusion line) will remain in place until the 24-hour sample is taken.

During infusion, vital signs (including, if possible, supine diastolic and systolic blood pressure, pulse rate, and temperature) must be monitored pre-infusion, every 15 minutes (± 5 minutes) until the end of the infusion, and thereafter, every 30 minutes (± 10 minutes) until the infusion line is removed. Vital signs during the infusion are not required to be captured in the eCRF unless abnormalities are observed.

LMB-100 drug product (DP) should be administered diluted using a side flow set-up at 1:10 (0.1 mg/mL DP). In order to not compromise drug product physico-chemical stability, the dilution with

0.9% NaCl should be done **in line**, immediately prior to administration of the neat DP. The infusion duration should be 30 minutes (± 5 minutes), **however the duration can be increased at the discretion of the investigator** based on the total dose and volume to be administered and the patient's physical condition. Syringe preparation and infusion duration should not exceed a maximum of 4 hours. In case of any adverse events related to the infusion, please refer to the specific recommendation described in section 3.3.2.

3.2.1.3 Premedications for Patients Receiving LMB-100

Due to the prevalence of infusion related reactions (IRRs) seen in the previous study of LMB-100, all patients will be predicated 30-60 minutes (+ 30 minutes) prior to each LMB-100 administration with the following medications:

- Diphenhydramine 25-50 mg PO or IV
- Ranitidine 150 mg PO
- Acetaminophen 650 mg PO
- Dexamethasone 20 mg, PO, 6-12 hours prior to LMB-100 administration, OR
- Dexamethasone 10 mg, IV, 30 – 60 minutes (+30 minutes) prior to LMB-100 administration, OR
- equivalent dose of another corticosteroid as clinically indicated

(See section 3.2.2 for complete instructions on response to IRRs)

Participants who experienced an IRR of Grade 3 or 4 on a previous infusion where dexamethasone or another steroid was pre-administered should not receive further LMB-100 and will be discontinued from study therapy.

Table 11 - Premedication for LMB-100

	Dose (mg)	Route
Acetaminophen	650	Orally
Ranitidine	150	Orally
Diphenhydramine[a]	25-50	Orally or IV
Dexamethasone[b]	10	IV
	OR	
Dexamethasone[b]	20	Orally

[a] or alternative antihistamine at an adequate dose.

[b] or equivalent dose of another corticosteroid administered if a >grade 2 IRR occurred on a previous infusion despite standard pre-medication.

3.2.2 Nab- paclitaxel (Arms B1 and B2 Only)

Nab-paclitaxel will be administered at a dose of 100 mg/m^2, intravenously over 30 minutes on Days 1 and 8 of each 21 day cycle for up to 6 cycles in the absence of toxicity or disease progression.

Detailed information about the preparation of the infusion solution and administration of *nab*-paclitaxel including information about compatible infusion bags, administration sets, and in line filters can be found in the package insert.

3.2.3 Co-administration of Nab-paclitaxel and LMB-100 (Arms B1 and B2 only)

On days when both LMB-100 and *nab*-paclitaxel are both scheduled to be administered (Day 1 of cycles 1 and 2), *nab*-paclitaxel will be administered first. LMB-100 infusion will be initiated 30 minutes after completion of the *nab*-paclitaxel.

3.3 DOSE MODIFICATIONS

3.3.1 For Arm A1 Subjects Treated at 170 mcg/kg only

As discussed in section 1.2.3.5, the three subjects treated at 170 mcg/kg will be subsequently dosed at 140 mcg/kg. Any further adverse events will be managed as per Table 12 below.

3.3.2 For all Subjects

Table 12 below provides a guideline on how to manage certain toxicities which are expected with LMB-100 based on preclinical studies and previous clinical experience with molecules in the same class

Table 12 - Guidelines for Managing Specific LMB-100 Adverse Events

Event	Action to Be Taken
IRR/hypersensitivity reaction	If an IRR/hypersensitivity develops, the infusion of LMB-100 should be temporarily slowed down or interrupted. The patient should be monitored until complete resolution of the symptoms and treated as clinically indicated. Treatment or concomitant medication may include acetaminophen, antihistamine, IV saline, oxygen, bronchodilators, corticosteroids, and vasopressors depending on the symptoms. If the infusion is interrupted: ○ In the event of IRR CTCAE Grade1, upon resolution of symptoms, the infusion will resume at the same rate (the rate being used at the time that the IRR occurred). ○ In the event of IRR Grade 2 or 3, upon resolution of symptoms, the infusion will resume at one-half the previous rate. The infusion can be re-escalated to initial rate if considered well tolerated after 1 hour of infusion. ○ In the event of IRR CTCAE Grade 3, or CTCAE Grade 4 (which may include pulmonary or cardiac events) or an anaphylactic reaction: ▪ The infusion must be stopped and the patient should receive aggressive treatment

Event	Action to Be Taken
	○ Patients experiencing IRR CTCAE Grade 4 or anaphylaxis must be permanently discontinued from LMB-100 treatment
Vascular leak syndrome	In the event of Grade ≥2 CTCAE vascular leak syndrome (medical intervention indicated): ○ Delay LMB-100 administration until complete resolution of the event ○ For hypotension minimize fluid resuscitation to avoid fluid overload Minimize crystalloid solutions (e.g., saline) ○ Vasopressor support (e.g., phenylephrine) if indicated to stabilize blood pressure ○ Administer colloidal solutions (e.g., albumin) if there is a clinically significant and persistent systolic blood pressure drop, and the patient is symptomatic, or urine output declines ○ For pulmonary congestion provide diuretic and/or albumin treatment in case of hypoalbuminemia as appropriate ○ Progressive shortness of breath may require in addition endotracheal intubation or drainage of a pleural effusion ○ For oliguria and /or rising serum creatinine level delay LMB-100 if Grade C3 urine output (<10 mL/hr) ○ Use fluids judiciously if increase in urine output is required ○ Use dopamine if patient is unresponsive to or unable to tolerate fluids Monitor serum albumin levels prior to each LMB-100 treatment cycle ○ In the event of Grade ≥2 CTCAE pericardial effusion (asymptomatic effusion small to moderate size), consider delaying LMB-100 administration. In the event of Grade ≥3 CTCAE pericardial effusion (effusion with physiologic consequences) stop LMB-100 treatment until full resolution
Inflammatory reactions to serosal membranes	○ Hydrocortisone (200 mg IV) or equivalent dose of another corticosteroid as clinically indicated ○ In the event of Grade 2 CTCAE pericardial effusion (asymptomatic effusion small to moderate size), consider delaying LMB-100 administration. In the event of Grade ≥3 CTCAE pericardial effusion (effusion with

Event	Action to Be Taken
	physiologic consequences) stop LMB-100 treatment until full resolution o In the event of pleuritis resulting in mild to severe pleuritic pain, treat with analgesics or steroids as clinically indicated o For patients who have previously experienced pleuritis consider administration of a tapering course of prednisone for 7 days starting with the next LMB-100 infusion
Renal Toxicity	Periodic monitoring of renal function and serum electrolytes IV fluids 0.9 NaCl may be administered after LMB-100 infusion if clinically indicated because of increasing serum creatinine or decreased urine output.
IRR = infusion related reaction; IV = intravenous; CTCAE = Common Terminology Criteria for Adverse Events	

3.3.3 Nab-paclitaxel

If *nab*-paclitaxel is withdrawn prior to cycle 2, LMB-100 may still be continued in the absence of progressive disease.

Dose modifications of *nab*-paclitaxel for myelosuppression and other chemotherapy related toxicities will be made as per the NSCLC instructions in the package insert.

3.4 STUDY CALENDAR

3.4.1 Arms A1 and A2 Calendar

1 cycle =21 days

Screening assessments must occur within 28 days prior to enrollment. If screening assessments are performed within 3 days prior to dosing, the assessments do not need to be repeated on C1D1 unless otherwise indicated (see ECG and pregnancy test below).

Assessments after C1D1 may be performed up to 3 days prior to indicated time unless otherwise indicated.

Dosing cycles after cycle 1 may be delayed for up to two weeks to accommodate schedule conflicts, Federal holidays and inclement weather, etc.

		Cycle 1					**Subsequent Cycles**					**Post Therapy Follow-up**	
Procedure	**Screening**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Follow-Up Visit[13]**	**Long-Term Follow Up**
LMB-100		X	X	X			X	X	X				
History and PE	X	X	X	X	X	X	X	X	X	C2 only		X	
Weight		X	X	X	X	X	X	X	X	C2 only		X	
Height		X											
Vital signs[1]	X	X	X	X	X	X	X	X	X	C2 only		X	
Performance Score	X	X					X						
Labs[2]	X	X	X	X	X	X	X	X	X	X		X	
Urinalysis	X	X					X						
HLA Typing (Class I and Class II)		X[3]											
Anti HIV, anti HCV, HBSAg	X												
Urine or serum hCG in women of childbearing potential[4]	X	X[5]					X					X	
Confirmation of dx[6]	X												
NIH Advance Directives Form		X[7]											

Procedure	Screening	Cycle 1					Subsequent Cycles					Post Therapy Follow-up	
		Day 1	Day 3	Day 5	Day 8	Day 15	Day 1	Day 3	Day 5	Day 8	Day 15	Follow-Up Visit[13]	Long-Term Follow Up
Biopsy (optional)		X[8]					C3 only[9]						
Correlative Research Studies		Please see section 5.2											
PK		Please see section 5.2.1											
CT CAP and/or if clinically indicated MRI +/- gadolinium	X	Every 6 weeks ± 7 days										X[10]	X[14]
FDG-PET	X	Every 6 weeks ± 7 days										X[10]	X[14]
ECG[11]	X	X	X	X			X	X	X			X	
Echocardiogram	X												
Chest X-Ray[12]	X			X			X						
Annual phone call to monitor survival and additional CA therapy													X
Adverse Events		Monitored continuously											
Concomitant Medications		Monitored continuously											

1 **At screening:** heart rate, blood pressure, body temperature, pulse oximetry. **During the infusions** of all study medication in Cycles 1 and 2, vital signs (heart rate, blood pressure, body temperature) have to be monitored pre-infusion and every 15 minutes (± 5 minutes) during the infusion and then every 30 minutes (± 10 minutes) from the end of the infusion until the infusion line is removed. From Cycle 3 onwards, vital signs have to be monitored only pre and post infusion if study medication has been tolerated well in previous cycles.

2 CBC with differential, Acute Care Panel, Hepatic Panel, Mineral Panel, creatine kinase, C-reactive protein, PT, PTT, fibrin degradation products.

3 May be performed after study consent is signed but prior to treatment initiation (baseline)

4 Required in women of childbearing potential; i.e. premenopausal women and women ≤ 2 years after menopause (menopause is defined as amenorrhea for > 2 years.

5 Only required if more than 14 days have passed since screening.

6 Please see section 2.2 for tissue requirements

7 As indicated in section 12.3, all subjects will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

8 To be performed at baseline – any time after patient has signed the study consent, but before study therapy initiation.

9 To be performed after the completion of 2 cycles of study therapy.

[10] Performed only if patient removed from study therapy for reason other than progressive disease

[11] Single 12-lead ECG will be recorded at screening (within 7 days before first dose of study treatment), then pre- and end of LMB-100 infusion for first 3 cycles and at the withdrawal and follow-up visit. Pre-infusion at all other study drug administrations. Additional unscheduled ECG assessments should be performed if cardiovascular symptoms or abnormalities occur.

[12] Chest X-rays will be obtained at screening and pre-infusion time on indicated dosing days. In addition, Chest X-ray will be performed when clinically indicated during the study treatment period. Chest X-rays will be obtained at the time of the tumor assessments, when convenient

[13] The assessments listed refer to those that will be performed if the patient is seen in clinic 4- 6 weeks after the last dose of study drug. If the patient is unable to return to the clinic for the follow up visit, an adverse event assessment will be performed by telephone.

[14] Scans performed only in patients who have not had progressive disease. Scans will continue every 6 weeks until disease progression.

3.4.2 Arms B1 and B2 Calendar

1 cycle =21 days

Screening assessments must occur within 28 days prior to enrollment. If screening assessments are performed within 3 days prior to dosing, the assessments do not need to be repeated on C1D1 unless otherwise indicated (see ECG and pregnancy test below).

Assessments after C1D1 may be performed up to 3 days prior to indicated time unless otherwise indicated.

Dosing cycles after cycle 1 may be delayed for up to two weeks to accommodate schedule conflicts, Federal holidays and inclement weather, etc.

		Cycle 1 and cycle 2					**Subsequent Cycles**					**Post Therapy Follow-up**	
Procedure	**Screening**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Follow-Up Visit[13]**	**Long-Term Follow Up**
LMB-100		X	X	X									
Nab-paclitaxel		X			X		X			X			
History and PE	X	X	X	X	X	C1 only	X			X		X	
Weight		X	X	X	X	C1 only	X			X		X	
Height		X											
Vital signs[1]	X	X	X	X	X	C1 only	X			X		X	
Performance Score	X	X					X						
Labs[2]	X	X	X	X	X	C1 only	X			X		X	
Urinalysis	X	X					X						
HLA Typing (Class I and Class II)		C1 only [3]											
Anti HIV, anti HCV, HBSAg	X												
Urine or serum hCG in women of childbearing potential[4]	X	X[5]					X					X	
Confirmation of dx[6]	X												
NIH Advance Directives Form		X[7]											

Procedure	Screening	Cycle 1 and cycle 2					Subsequent Cycles					Post Therapy Follow-up	
		Day 1	Day 3	Day 5	Day 8	Day 15	Day 1	Day 3	Day 5	Day 8	Day 15	Follow-Up Visit[13]	Long-Term Follow Up
Biopsy (optional)		X[8]					C3 only[9]						
Correlative Research Studies		Please see section 5.2.2											
PK		Please see section 5.2.2											
CT CAP and/or if clinically indicated MRI +/- gadolinium	X	Every 6 weeks ± 7 days										X[10]	X[14]
FDG-PET	X	Every 6 weeks ± 7 days										X[10]	X[14]
ECG[11]	X	X	X	X			X					X	
Echocardiogram	X												
Chest X-Ray[12]	X	C2 only		C1 only									
Annual phone call to monitor survival and additional CA therapy													X
Adverse Events		Monitored continuously											
Concomitant Medications		Monitored continuously											

1 **At screening:** heart rate, blood pressure, body temperature, pulse oximetry. **During the infusions** of all study medication in Cycles 1 and 2, vital signs (heart rate, blood pressure, body temperature) have to be monitored pre-infusion and every 15 minutes (± 5 minutes) during the infusion and then every 30 minutes (± 10 minutes) from the end of the infusion until the infusion line is removed. From Cycle 3 onwards, vital signs have to be monitored only pre and post infusion if study medication has been tolerated well in previous cycles.

2 CBC with differential, Acute Care Panel, Hepatic Panel, Mineral Panel, creatine kinase, C-reactive protein, PT, PTT, fibrin degradation products.

3 May be performed after study consent is signed but prior to treatment initiation (baseline)

4 Required in women of childbearing potential; i.e. premenopausal women and women ≤ 2 years after menopause (menopause is defined as amenorrhea for > 2 years.

5 Only required if more than 14 days have passed since screening.

6 Please see section 2.2 for tissue requirements

7 As indicated in section 12.3, all subjects will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

8 To be performed at baseline – any time after patient has signed the study consent, but before study therapy initiation.

[9] To be performed after the completion of 2 cycles of study therapy.

[10] Performed only if patient removed from study therapy for reason other than progressive disease

[11] Single 12-lead ECG will be recorded at screening (within 7 days before first dose of study treatment), then pre- and end of LMB-100 infusion for the first 2 cycles and at the withdrawal and follow-up visit. Pre-infusion at all other study drug administrations. Additional unscheduled ECG assessments should be performed if cardiovascular symptoms or abnormalities occur.

[12] Chest X-rays will be obtained at screening and pre-infusion time on indicated dosing days. In addition, Chest X-ray will be performed when clinically indicated during the study treatment period. Chest X-rays will be obtained at the time of the tumor assessments, when convenient

[13] The assessments listed refer to those that will be performed if the patient is seen in clinic 4- 6 weeks after the last dose of study therapy. If the patient is unable to return to the clinic for the follow up visit, an adverse event assessment will be performed by telephone.

[14] Scans performed only in patients who have not had progressive disease. Scans will continue every 6 weeks until disease progression.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.5.1 Criteria for removal from protocol therapy

Prior to removal from study, a safety follow up visit as indicated in section 3.5.2 must be completed.

- Progressive disease
- Arm A1 or A2 participant has completed 4 cycles of study therapy
- Arm B1 or B2 participant has completed 6 cycles of study therapy
- Participant requests to be withdrawn from active therapy
- Pregnancy
- Unacceptable Toxicity as defined in sections 3.1.1 and 3.3
- Investigator discretion

3.5.2 Follow Up

All patients will be asked to return for a follow up visit ~ 4 – 6 weeks after the last dose of the study drug (see Study Calendar for assessments). If the subject is unable to return to the NIH, a brief assessment for adverse events will be performed by telephone. Subjects who have not had progressive disease will continue to have follow-up scans every 6 weeks until disease progression.

Thereafter, all patients or their physicians will be contacted annually by telephone to assess survival status and additional anti-cancer therapy.

3.5.3 Off-Study Criteria

- Patient lost to follow-up
- Participant requests to be withdrawn from study+
- Death

+ Patient will be required to complete follow up visit prior to removal from study therapy for this reason.

3.5.4 Off Protocol Therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1.1 Permitted Therapy

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter (OTC) drugs, approved dietary and herbal supplements, nutritional supplements) used by a patient from

the screening period until the follow-up visit. If any treatment is given within 4 weeks prior to screening this should be reported to the investigator and recorded in the eCRF.

All therapy and/or medication administered to manage adverse events should be recorded on the Adverse Event eCRF

4.1.2 Prohibited Therapy

Patients should be treated for all concomitant conditions and adverse events according to accepted standards of medical care at the discretion of the investigator. The following treatments are not permitted during the study:

- Any other investigational therapy
- Cytotoxic chemotherapy agents other than study agents
- Radiotherapy. Note: palliative 8Gy radiotherapy is allowed.
- Immunosuppressive therapy and chronically administered glucocorticoids (high dose is considered as > 20 mg of dexamethasone a day [or equivalent)] for > 7 consecutive days)
- Other systemic anti-neoplastic agents and targeted therapies

If any anti-neoplastic or investigational therapies listed above are needed, the patient will be considered to have evidence of progressive neoplastic disease and have experienced treatment failure with study treatment and should be withdrawn from study treatment.

All concomitant treatments must be documented in the eCRF.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Pharmacokinetic Assessments

All blood samples for PK assessment will be collected from an IV line different to that receiving the infusion to measure free and total concentrations for LMB-100 for all patients. The date and time of each sample collection will be recorded. If multiple samples are drawn at a given time point, the PK sample should take precedence.

Free and total plasma concentrations of LMB-100 will be measured using validated ligand-binding assays.

5.1.1.1 Sample collection:

Blood will be collected in 2 mL K_2EDTA tubes (purple top) at the times defined in section 5.2. Samples should be inverted 8 to 10 times after collection. Store on wet ice or at 4°C. Processing within 60 minutes of blood collection is highly preferred.

5.1.1.2 Sample processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the CPP the following procedures should be followed:

1. Store on wet ice until centrifugation.
2. Centrifuge 1500xg for 10 minutes at 4°C within 60 minutes of blood collection.
3. Transfer plasma specimen to 2mL cryovials and store at -70°C.

The analyses will be performed retrospectively in batched samples or at the end of the trial.

5.1.1.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to Dr. Jon Inglefield in Frederick for analysis.

Leidos Biomedical, Inc.
Attention: Ms. Yanyu Wang, Dr. Jon Inglefield
Building 469, Room 120
Miller Drive
Frederick, MD 21702
Phone: 301-846-6905/301-846-6865

5.1.1.4 Sample storage

Samples will be stored in the CPP until shipment to the Leidos Biomedical Inc. Lab in Frederick.

5.1.2 Assessment of anti-drug antibodies (ADAs)

5.1.2.1 Sample Collection

Samples will be collected before first dose of LMB-100 administration during each cycle in which LMB-100 is administered (See section 5.2)

Draw 2mL into K_2EDTA tube (purple top). Samples should be inverted 8 to 10 times after collection. Store on wet ice or at 4°C. Processing within 60 minutes of blood collection is highly preferred.

5.1.2.2 Sample Processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the CPP, each sample should be processed in the following manner:

1. Store on wet ice until centrifugation.

2. Centrifuge 1500xg for 10 minutes at 4°C within 60 minutes of blood collection.
3. Transfer plasma specimen to 2mL cryovials and store at -70°C.

Autoantibody levels will be retrospectively assessed.

5.1.2.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to Dr. Jon Inglefield in Frederick for analysis.

Leidos Biomedical, Inc.
Attention: Ms. Yanyu Wang, Dr. Jon Inglefield
Building 469, Room 120
Miller Drive
Frederick, MD 21702
Phone: 301-846-6905/301-846-6865

5.1.2.4 Sample Storage

Samples will be stored in the CPP until shipment to the Leidos Biomedical Inc. Lab in Frederick.

5.1.3 Mesothelin and Megakaryocyte Potentiating Factor (MPF) Serum Samples:

The levels of serum mesothelin as well as megakaryocyte potentiating factor, which is released into serum from the processing of mesothelin precursor protein will be assessed in order to determine correlation with therapeutic response.

5.1.3.1 Sample Collection

Samples will be obtained as follows:

- Arms A1 and A2 - prior to the first LMB-100 dose of each cycle and at the end of treatment
- Arms B1 and B2 -prior to the first LMB-100 dose of cycles 1 and 2, prior to the first *nab*-paclitaxel administration during cycles 3 – 6, and at the end of treatment.

All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (2mL) will be drawn into a 3.5-mL serum separator tube (tiger top tube) labeled as follows:

- Subject ID Number
- Study Number
- Time and date of collection

5.1.3.2 Sample Processing

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the CPP, each sample should be processed in the following manner:

Allow blood to clot for 10 minutes and centrifuge to separate the serum within 30 minutes of collection. If unable to process within 30 minutes, then whole blood tubes may be stored upright in refrigerator (4-8°C) for up to 48 hours prior to processing. Processing of samples within 30 minutes is strongly preferred. Stability studies will establish if degradation of soluble mesothelin in whole blood during 0.5 to 48 hours is significant and therefore if the data from these samples should be included in the analysis.

Transfer the serum into two pre-labeled cryotubes and immediately freeze by placing on dry ice. Transfer frozen serum samples into a – 80°C freezer for storage.

5.1.3.3 Sample Storage

All serum samples will be stored by Dr. Figg's Clinical Pharmacology Program.

5.1.4 Retrospective Analysis of Mesothelin Expression in tumor tissue

IHC analysis will be performed by the Laboratory of Pathology at NCI to determine mesothelin expression within the tumor at any time after study enrollment. Leftover tissue from archival specimens or tumor biopsies obtained at screening or from optional collections at baseline or after cycle 2 may be used for this purpose. Specimens will be used to correlate treatment with response with mesothelin expression in an exploratory analysis.

5.1.4.1 Specimen collection

Collection of optional tumor biopsies should be guided by ultrasound, CT scan, or other method according to the location of the selected lesion using a ≤ 18 gauge needle to provide cores ideally of at least 20 mm in length or equivalent size. At least 2, ideally 4 core biopsies will be obtained at each time point (baseline and after cycle 2). Fine needle aspiration and biopsy of bone lesions are not acceptable. All biopsies collected under this protocol will undergo review in the NCI Laboratory of Pathology.

5.1.5 Cytokines for identification of mechanism for PE-mediated capillary leak syndrome

PE-based RITs cause dose-limiting CLS. At low doses CLS manifests as mild and transient weight gain, hypoalbuminemia, and peripheral or facial edema. At higher doses it can cause life-threatening cardiopulmonary compromise. Previous studies in rats have indicated that pathological changes indicative of CLS onset occur within just two hours of toxin administration and even when the PE fragment lacks a targeting domain.[32] In vitro studies with cultured endothelial cells have demonstrated that super-physiologic doses of PE-based RITs cannot induce endothelial cell toxicity unless the cells express the RIT target.[33] Together these data suggest the hypothesis that **PE-based RITs cause CLS by triggering release of vasoactive cytokines by specific immune cells rather than through direct damage to endothelial cells**.

5.1.5.1 Specimen collection

To test this hypothesis, we will collect additional serum from participants during each of LMB-100 therapy on:

- Days 1, 3, and 5 (Pre-dose)
- Day 8

in 4 mL red SST tubes

5.1.5.2 Sample processing

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Cytokine levels will be retrospectively assessed.

5.1.6 Gene expression-based characterization of the immune landscape before and after treatment with LMB-100

As referenced above, optional tumor biopsy will be performed in consenting patients when deemed feasible at the following time points: before initiation of therapy (archival tissue may also be used) and after two cycles of therapy. We will evaluate tumor biopsies before and after treatment with LMB-100 (using samples referenced in section above) using a hybridization-based digital gene expression platform nCounter (NanoString Technologies). This platform allows for unbiased multiplexed quantification of RNA transcripts achieving sensitivity comparable to quantitative reverse-transcription polymerase chain reaction (Q-RT-PCR), without any enzymatic reaction involved in the process. We have expertise in the use of this technology for the characterization of purified cell subsets and also for the study of the changes that occur in human tumors, as a consequence of a given treatment, using tumor core biopsy tissues. This analysis can be performed on flash frozen biopsies without any further isolation or enrichment of specific cell types.

For the present study, we will screen pre- and post-treatment samples for the expression of markers of immune cell subsets (CD3, CD8, CD4, etc.), local production of cytokines (interferon-gamma, tumor necrosis factor-alpha, etc.) and chemokines (CXCL13, CCL5, etc.), adhesion molecules and others. The purpose of this study is to characterize the molecular changes that occur within the tumors following treatment with LMB-100. The ultimate goal is to gain a better understanding of the mechanism of action of this treatment, and to identify molecular correlates of clinical outcomes such as objective responses and/or improved survival.

In order to achieve these goals, flash frozen samples will be subjected to total RNA isolation followed by hybridization with capture and detection probes specific for 620 transcripts, including genes involved in the regulation of the immune function and also markers expressed by tumor cells and tumor stroma. To cover those target transcripts, a combination of a commercially available pre-designed probe set (GX Human Immunology v2, NanoString Technologies) will be used in combination with a custom-designed code set of thirty additional targets (Panel Plus, NanoString Technologies). Hybridization complexes will be quantified using a NanoString nCounter Analysis System, at the Genomics Core Facility of the Center for Cancer Research, NCI.

Results obtained by this approach will be correlated with data obtained from immunohistochemistry of tumor biopsies as well as with data from analysis of peripheral blood

populations, for a comprehensive study of the mechanism of action of LMB-100 in mesothelioma patients.

Samples will be stored in the Laboratory of Dr. Raffit Hassan, Building 10, Room 3B51.

5.1.7 Mapping T cell epitopes in LMB-100.

Identification and investigation of T cell epitopes in therapeutic proteins is important for understanding of the immunogenicity response against LMB-100. Identification of those T cell epitopes will allow us to engineer the next generation of immunotoxin which will be less immunogenic because it will have less T cell epitopes.

Method: For T cell epitope mapping we isolate PBMC from patients that were previously treated with the immunotoxin and had neutralizing antibodies. PBMC are frozen at a concentration of 1.5 $x10^7$ cells/ml/vial. Cells are viably frozen in the following freezing media: RPMI, 10% human AB serum, 1% P/S (antibiotic) and 7.5% DMSO.

The PBMC are stimulated with the whole protein and the activated T cells are expanded using human IL2 which is a lymphocyte stimulator. Next, the in vitro expanded PBMC are re- stimulated with a peptide library spanning the sequence of the Fab portion of the immunotoxin. The T cell activation is detected by monitoring secretion of IL2 using IL2 ELISpot.

An illustration of the method, the controls and threshold determination were previously described in Mazor et al 2012.[20]

5.1.7.1 Sample collection

Samples will be collected at baseline and at the end of treatment in eight 8 mL whole blood tubes (black and blue)." [BD Vacutainer® CPT (REF 362761)]

5.1.7.2 Sample processing

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

PBMCs will be processed within 6 hours post-collection using Ficoll- Hypaque density-gradient separation according to manufacturer's instructions. Collect the buffy coat and wash three times with Dulbecco's PBS without Ca and Mg. Cryopreserve in liquid nitrogen at a concentration of 1.5 x 10^7 cells/mL in RPMI1640 supplemented with 5% human AB serum and 7.5% DMSO.

5.1.7.3 Sample storage

Samples will be stored in by the Clinical Pharmacology Program; analyses will be performed in the laboratory of Dr. Raffit Hassan.

5.1.8 Assessment of Myeloid Derived Suppressor Cells (MDSC) in Subjects with Mesothelioma

5.1.8.1 Sample collection

Samples will be collected at baseline prior to first dose of LMB-100 and the end of cycle 2 and at the end of treatment. All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (30 mL) will be drawn into three 10 mL sodium heparin (green top) tubes.

The tube should be labeled with the following information:

- Subject ID Number
- Study Number
- Time and date of collection
-Specimen type: **peripheral blood**

5.1.8.2 Sample processing

The samples will be sent to NIH flow lab located at 3S240, South part of Building 10.

All specimens for flow cytometry will be scheduled in advance by contacting Dr. Stetler-Stevenson stetler@mail.nih.gov and/or Dr. Yuan yuanc@mail.nih.gov by email. Peripheral blood samples should arrive no later than 1 pm to the flow cytometry laboratory on the day scheduled.

Specimens are received in the laboratory via escort or via delivery by someone from the clinical team. Specimens for delivery AFTER HOURS ONLY may also be placed in the secure specimen drop box located in Laboratory of Pathology 3N hallway. Peripheral blood specimens are collected in sodium heparin tubes, and are stored at room temperature until delivery.

The specimens should be delivered as soon as possible after collection, and cannot be evaluated if > 48 hours old.

Upon receipt in the flow lab, the sample will be checked for correct labeling with appropriate patient identifiers and specimen type (e.g. peripheral blood).

5.2 SAMPLE COLLECTION SCHEDULE

5.2.1 Arms A1 and A2 sample collection schedule

Cycle	**Day**	**PK** (5.1.1) 2 mL K_2EDTA tube	**ADA** (5.1.2) 2 mL K_2EDTA tube	**Serum mesothelin and MPF** (5.1.3) 2 mL blood in 3.5 mL tiger top tube	**PE mediated CLS (Cytokines)** (5.1.5) 4 mL red SST tube	**T-cell epitopes** (5.1.7) Eight 8 mL whole blood tubes	**MDSC Assessment** (5.1.8) Three 10 mL sodium heparin (green top) tubes	**Tumor Sample**[a] (5.1.4,5.1.6) NA
Screening	Screening period ± 3 days							X[a]
1	1	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion	Pre-dose	X	Pre-dose	X[b]	X[b]	X[c]
	3				Pre-dose			
	5	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion			Pre-dose			
	8				X			

Cycle	**Day**	**PK** (5.1.1) 2 mL K_2EDTA tube	**ADA** (5.1.2) 2 mL K_2EDTA tube	**Serum mesothelin and MPF** (5.1.3) 2 mL blood in 3.5 mL tiger top tube	**PE mediated CLS (Cytokines)** (5.1.5) 4 mL red SST tube	**T-cell epitopes** (5.1.7) Eight 8 mL whole blood tubes	**MDSC Assessment** (5.1.8) Three 10 mL sodium heparin (green top) tubes	**Tumor Sample**[a] (5.1.4,5.1.6) NA
2	1	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion	Pre-dose	X	Pre-dose			
	3				Pre-dose			
	5	Pre-dose, EOI			Pre-dose			
	8				X			
3 & 4	1	Pre-dose and EOI	Pre-dose	X	Pre-dose		X (end of cycle 2)	X[c]
	3				Pre-dose			
	5	Pre-dose and EOI			Pre-dose			
	8				X			
At time of Progression			X					
End of Treatment				X		X	X	

Cycle	**Day**	**PK** (5.1.1)	**ADA** (5.1.2)	**Serum mesothelin and MPF** (5.1.3)	**PE mediated CLS (Cytokines)** (5.1.5)	**T-cell epitopes** (5.1.7)	**MDSC Assessment** (5.1.8)	**Tumor Sample**[a] (5.1.4,5.1.6)
		2 mL K_2EDTA tube	2 mL K_2EDTA tube	2 mL blood in 3.5 mL tiger top tube	4 mL red SST tube	Eight 8 mL whole blood tubes	Three 10 mL sodium heparin (green top) tubes	NA
Follow-up Visit			X					

ADA=anti-drug antibody; EOI=End of infusion; PK=pharmacokinetic; MDSC= myeloid derived suppressor cell

a. Archival or if not available, a fresh Biopsy or Tumor Effusion (mandatory). Even if archival sample is available, patient may be asked for optional biopsy sample.

b. May be collected prior to day 1 at any time after the consent is signed (baseline).

c. Optional biopsies – collected at baseline (biopsy if collected at screening can be used as baseline sample) and at any time after the completion of 2 cycles of therapy.

5.2.2 Arms B1 and B2 Sample Collection Schedule

Cycle	**Day**	**PK** (5.1.1) 2 mL K_2EDTA tube	**ADA** (5.1.2) 2 mL K_2EDTA tube	**Serum mesothelin and MPF** (5.1.3) 2 mL blood in 3.5 mL tiger top tube	**PE mediated CLS (Cytokines)** (5.1.5) 4 mL red SST tube	**T-cell epitopes** (5.1.7) Eight 8 mL whole blood tubes	**MDSC Assessment** (5.1.8) Three 10 mL sodium heparin (green top) tubes	**Tumor Sample**[a] (5.1.4,5.1.6) NA
Screening	Screening period ± 3 days							X[a]
1	1	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion	Pre-dose	X	Pre-dose	X[b]	X[b]	X[c]
	3				Pre-dose			
	5	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion			Pre-dose			
	8				X			
2	1	Pre-dose, EOI, 1, 2, 3, 4, and 6 hours after start of infusion	Pre-dose	X	Pre-dose			
	3				Pre-dose			

Cycle	**Day**	**PK** (5.1.1) 2 mL K_2EDTA tube	**ADA** (5.1.2) 2 mL K_2EDTA tube	**Serum mesothelin and MPF** (5.1.3) 2 mL blood in 3.5 mL tiger top tube	**PE mediated CLS (Cytokines)** (5.1.5) 4 mL red SST tube	**T-cell epitopes** (5.1.7) Eight 8 mL whole blood tubes	**MDSC Assessment** (5.1.8) Three 10 mL sodium heparin (green top) tubes	**Tumor Sample**[a] (5.1.4,5.1.6) NA
	5	Pre-dose, EOI			Pre-dose			
	8				X			
3 -6	1			X			X (end of cycle 2)	X[c]
	3							
	5							
	8							
At time of Progression			X					
End of Treatment				X		X	X	
Follow-up Visit			X					

ADA =anti-drug antibody; EOI =End of infusion; PK =pharmacokinetic; MDSC= myeloid derived suppressor cell

a. Archival or if not available, a fresh Biopsy or Tumor Effusion (mandatory). Even if archival sample is available, patient may be asked for optional biopsy sample.

b. May be collected prior to day 1 at any time after the consent is signed (baseline).

c. Optional biopsies – collected at baseline (biopsy if collected at screening can be used as baseline sample) and at any time after the completion of 2 cycles of therapy.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.3.1 Clinical Pharmacology Program

All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Labmatrix utilized by the CPP. . This is a secure program, with access to t Labmatrix limited to defined CPP personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle, time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system.. For each sample, there are notes associated with processing method (delay in sample processing, storage conditions on the ward, etc.)

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20°C or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following OHSRP/IRB approval of an additional protocol, granting the rights to use the material or if the use is not considered to be human subjects research.

5.3.2 Leidos Biomedical, Inc. Lab

Blood and tissue collected during the course of this study will follow storage, handling and labeling procedures to ensure that security, confidentiality and sample integrity are maintained. All samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Thus samples will be coded, with access to code key linking to personal data restricted to the study investigators.

All cryopreserved samples are tracked for freezer location and storage criteria. All Samples are stored in a locked freezer at -70°C according to stability requirements. These freezers are located offsite at NCI-Frederick, at the Leidos Biomedical, Inc. Lab in Frederick, MD. Samples will be

stored until requested by a researcher named on the protocol. All use and requests for use will be recorded by the Leidos Biomedical, Inc. Lab. Any unused samples must be returned.

Some samples as indicated below may be stored in monitored freezers/refrigerators in the investigator's laboratory at specified temperatures with alarm systems in place.

At the completion of this protocol, samples will remain in storage as detailed above. If additional studies are to be performed on any samples retaining patient identifiers, obtained during the conduct of this trial, access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If specimens are to be discarded at any point, they will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested).

5.3.3 Laboratory of Dr. Raffit Hassan

This study will follow storage, handling and labeling procedures to ensure that security, confidentiality and sample integrity are maintained. All samples (blood or tissue) are tracked by distinct identification labels generated by Labmatrix that include a unique patient identifier and date of specimen collection. Thus samples will be coded, with access to the code key linking to personal data restricted to the study investigators.

Depending on specimen type, samples are stored in liquid nitrogen, in monitored freezers/refrigerators at either -20 or -80°C according to stability requirements or in a slide cabinet in the research Laboratory of Dr. Raffit Hassan (Building 10, Room 3B51)

5.3.4 NIH Flow Cytometry Laboratory

Specimens received in the flow cytometry laboratory are accessible only to the flow cytometry laboratory team, as the laboratory door is locked and key access is only provided to the flow cytometry laboratory members. Additionally, the flow cytometry laboratory is set behind a set of double doors that are accessible only by badge/key card access.

5.3.5 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open as long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or until a subject withdraws consent for their continued use, at which time they will be destroyed. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and patient consent or an exemption from OHSRP.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section 7.2.1.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Study data will be recorded and stored in the C3D and Labmatrix databases. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the study intervention was last administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section 7.2.1.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in BTRIS

_X_ Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names:__clinicaltrials.gov__.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

No large scale genomic data will be generated on this study; therefore, the NIH GDS policy does not apply.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 6 weeks (2 cycles). In addition to a baseline scan, confirmatory scans should also be obtained no less than 4 weeks following initial documentation of objective response.

Response and progression will be assessed by the investigator on the basis of physical examinations, computed tomography (CT) or Magnetic Resonance (MR) scans, and potentially other modalities according to standard of care.

For peritoneal mesothelioma, the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[34] will be used. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

For pleural mesothelioma, modified RECIST for MPM (malignant pleural mesothelioma)[35] should be used as described in section 6.3.3.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with LMB-100.

The dose determining population will consist of all patients evaluable for DLT. Patients are evaluable for DLT if they are evaluable for toxicity and discontinued earlier due to DLT or completed the DLT observation period and have undergone safety evaluations.

Evaluable for pharmacokinetic analysis: All patients that are evaluable for toxicity will be included in the PK analysis population. Patients will be excluded from the PK analysis population if they significantly violate the inclusion or exclusion criteria, deviate from the protocol, or if data are unavailable or incomplete which may influence the PK analysis. Excluded cases will be documented with the reason for exclusion.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Peritoneal Mesothelioma

6.3.2.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters)

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.2.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition

protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data, which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published.[36-38] In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.[39]

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression

occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.2.3 RECIST version 1.1 Response Criteria

6.3.2.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.2.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.2.3.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

n-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*

n-Target Lesions	New Lesions	Overall Response
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.3 Pleural Mesothelioma

Malignant pleural mesothelioma (MPM) lesions are difficult to measure reliably.[35] Therefore modified criteria were defined in 2004 adjusting target lesion measurements to the specific needs of this disease.

6.3.3.1 Modified RECIST Criteria for MPM

Target lesion:

Measurable at baseline and defined as tumor thickness measurements perpendicular to the chest wall or mediastinum in two positions at three separate levels on transverse cuts of CT scan. The sum of those 6 measurements define a pleural unidimensional measure. For reproducibility of lesion identification in follow up scans, cuts were taken at least 1 cm apart and close to anatomical landmarks in the thorax. Reassessments should be done at same position at the same level and by the same reader. Nodal, subcutaneous, and other measurable lesion were measured as per RECIST criteria. All unidimensional measurements were added to obtain total tumor measurement.

Evaluation of target lesions

- Complete Response (CR): Disappearance of all target lesions with no evidence of tumor elsewhere.
- Partial Response (PR): At least a 30% decrease in the total tumor measurement
- Confirmed response (PR and CR): require a repeat scan at least 4 weeks apart
- Progressive Disease (PD): At least a 20% increase in the total tumor measurement, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

6.3.3.2 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Response for this Category Also Requires:**
CR	CR	No	CR	≥4 wks. confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks. confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once ≥4 wks. from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

6.3.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.5 Progression-Free Survival

Progression free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.3.6 Objective Response Rate

Objective response rate (ORR) is defined as the proportion of patients with partial response or complete response.

6.3.7 Disease Control Rate

Disease control rate (DCR) is defined as the proportion of patients with stable disease, partial response or complete response

6.3.8 Overall Survival

Overall survival (OS) is defined as the duration of time from start of treatment to time of death

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING /IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section 7.2.1 will be submitted within the appropriate timelines..

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

Note: The protocol is no longer SMC review as of 9/17/2018 as no patients have been on study therapy since the time of the next scheduled review.

8 SPONSOR SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization

- A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs

occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section 6.1. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section 8.2.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Reporting Pregnancy

8.4.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section 8.1.2) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.4.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 3 months after the last dose of LMB-100 or 6 months after the last dose of *nab*-paclitaxel, whichever occurs later.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until the later or 3 months after the last dose of LMB-100 or 6 months after the last dose of nab-paclitaxel should, if possible, be followed up and documented.

8.5 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING PLAN

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6 and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

The primary objective of this phase I trial is to assess the safety and tolerability of LMB-100 with (Arms A1 and A2) and without (Arms B1 and B2) *nab*-paclitaxel in patients with malignant mesothelioma. Additional objectives are to determine the response rate at the RP2D, to retrospectively explore and describe the pharmacokinetic characteristics, including the number of effective cycles of LMB-100 therapy that can be given before the development of anti-LMB-100 neutralizing antibodies limits patient drug exposure.

10.1 ARMS A1 AND A2

The dose de-escalation study Arm A1 builds upon and extends the findings from the closed phase I trial of LMB-100 (NCT02317419) in which 0/4 patients treated at170 mcg/kg experienced a DLT and 2/4 patients treated at 250 mcg/kg experienced a DLT. As a result of these prior findings, it was planned that 6 patients would be treated at 170 mcg/kg (Table 7). If 0 or 1/6 patients have a DLT, then 170 mcg/kg would be declared the RP2D. If 2 or more of the 6 patients have a DLT, then 3 to 6 patients would be treated at 140 mcg/kg following the usual 3+3 design. 0 or 1 DLT in 6 patients at 140 mcg/kg will result in this level being the RP2D. Otherwise, dose levels of 100 mcg/kg or lower would be explored. Dose de-escalation below 140 was deemed unlikely given the available monotherapy data with LMB-100 (Table 1). It was considered likely that accrual to this phase would not need to exceed 12 patients. Patients treated at the RP2D in Arm A1 were to be included in the efficacy analyses at the RP2D.

For patients with malignant mesothelioma, low response rates are currently obtained with existing treatments. The goal of the dose expansion cohort would be to determine if using LMB-100 would rule out a 5% response rate and target a rate of 30%. The study will be conducted using a two-stage Minimax phase II trial design in order to rule out an unacceptably low 5% PR+CR rate (p0=0.05) in favor of an improved PR+CR response rate of 30% (p1=0.30). Both PR and CR will be considered a 'response' for purposes of this study. With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), this cohort was to have initially enrolled 13 evaluable patients and if 0 of the 13 have a response, then no further patients would be accrued. If 1 or more of the first 13 patients had a response, then accrual would continue until a total of 16 patients were enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there were 1 to 2 responses in 16 patients, this would be an uninterestingly low response rate. If there were 3 or more responses in 16 patients (18.8%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 51.3%.

After the enrollment of 10 evaluable patients the pharmacokinetic and anti-drug antibody level (ADA) data underwent an interim analysis. Dr. William Figg, who has expertise in pharmacokinetics, in conjunction with the NCI CCR leadership committee analyzed the PK data. Since the thresholds for ADA and its influence on pharmacokinetics are unclear, pre-specified criteria for making changes to the protocol were not established. The objective of the assessment was to make recommendations as to whether a therapeutic threshold has been reached and what, if any adjustments should be made with regard to the eligibility criteria and/or study plan. No adjustments were recommended as a result of the interim analysis.

With Amendment B, it was determined that the 170 mcg/kg dose would no longer be explored after DLT occurred in 3 of the first 3 subjects evaluated. The three existing patients were re-treated at the 140 mcg/kg dose and an additional 7 subjects were enrolled to be treated at 140 mcg/kg. Of 10 subjects, none experienced partial or complete response. Therefore, as of Amendment C, it is determined that patients will no longer be treated with LMB-100 monotherapy and all future subjects are to be enrolled on Arms B1 and B2.

10.2 ARMS B1 AND B2

The dose de-escalation study (Arm B1) plans to evaluate up to two dose levels of LMB-100 in combination with *nab*-paclitaxel. Once the RP2D has been determined, a dose expansion cohort (Arm B2) will be treated at the RP2D using the design discussed below. Patients treated at the RP2D in Arm B1 will be included in the efficacy analyses at the RP2D.

For patients with malignant mesothelioma, low response rates are currently obtained with existing treatments. The goal of the dose expansion cohort would be to determine if using LMB-100 would rule out a 5% response rate and target a rate of 30%. The study will be conducted using a two-stage Minimax phase II trial design in order to rule out an unacceptably low 5% PR+CR rate (p0=0.05) in favor of an improved PR+CR response rate of 30% (p1=0.30). Both PR and CR will be considered a 'response' for purposes of this study. With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), this cohort will initially enroll 13 evaluable patients and if 0 of the 13 have a response, then no further patients will be accrued. If 1 or more of the first 13 patients has a response, then accrual would continue until a total of 16 patients have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 responses in 16 patients, this would be an uninterestingly low response rate. If there were 3 or more responses in 16 patients (18.8%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 51.3%.

10.3 JUSTIFICATION OF ACCRUAL CEILING

The Arm B1 dose de-escalation cohorts may require up to 12 patients, and the expansion portion (Arm B2) may require up to 16 evaluable patients. However, with 6 patients from the Arm B1 dose de-escalation cohorts potentially being included in the analysis at the RP2D, up to 22 individual patients may be required. In order to allow for a small number of inevaluable patients in Arms B1 and B2 and adding the 10 patients already enrolled on the completed Arms A1 and A2, the accrual ceiling will be set at 34 patients. If 1-2 patients per month enroll on this trial, accrual would be expected to be completed in approximately 2.5 years.

11 COLLABORATIVE AGREEMENTS

11.1 CLINICAL TRIAL AGREEMENT

A Clinical Trial Agreement (CTA #01059-17) in place between the National Cancer Institute and Celgene for the provision of the *nab*-paclitaxel.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

LMB-100 is a mesothelin-targeted cFP and has shown preclinical dose-dependent activity in monotherapy and/or combination in xenografts representing MSLN-positive indications (NSCLC, mesothelioma, triple negative breast cancer, gastric cancer, pancreas, ovarian, potentially other tumor indications). The rationale to include MSLN- positive tumors of epithelial cell origin is based on preclinical models demonstrating promising anti-tumor efficacy. In addition, molecular pathology data demonstrated a high prevalence of mesothelin protein in these tumors. The

rationale to evaluate LMB-100 +/- *nab*-paclitaxel in advanced/metastatic mesothelioma is to preliminarily assess the safety and the anti-tumor activity of LMB-100 in this indication.

12.2 Participation of Children

There are no dosing or adverse event data are currently available on the use of LMB-100 with or without *nab*-paclitaxel in patients <18 years of age; therefore, children are excluded from this study.

12.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 12.5), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 Evaluation of Benefits and Risks/Discomforts

12.4.1 Risks from Study Drugs

Patient safety will be managed by careful proactive patient selection prior to study to exclude patients at risk from study treatment due to their pre-existing conditions. During the study, safety of patients will be proactively managed by protocol-mandated physical examinations, vital signs assessments, chest X-rays, ECGs, clinical laboratory assessments, and collection of adverse events and their assessment.

The risks of the study include those associated with study agent as discussed in section 13.

12.4.2 Radiation Risks

Subjects in this study may undergo up to 2 CT guided research biopsies which will result in exposure to approximately 1.5 rem. This amount is below the guideline of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee.

12.5 Risks/Benefits Analysis

Patients with advanced and/or metastatic mesothelioma, are in continuous need of improved therapy options. This is especially true for patients where no standard therapy exists such as the patient population that will be eligible for this trial. Preclinical data has demonstrated promising anti-tumor efficacy of LMB-100 in xenograft models in monotherapy and combination therapy.

Therefore, LMB-100 +/- *nab*-paclitaxel may improve clinical outcome of patients with mesothelioma. A number of clinically appropriate strategies to minimize risk to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefits of mesothelin targeted cFP for cancer patients retaining the ability to consent and those who lose capacity to consent during the course of the trial outweigh the risks associated with the proposed entry-into-human trial with LMB-100 +/- *nab*-paclitaxel.

12.6 Consent Process and Documentation

All patients will be thoroughly screened by the physician and the research nurse prior to completing the consent. During the initial consultation, the patient and family or friends, if present, will be presented with a forthright and detailed overview of the treatment option available to them at the NIH. The experimental nature of the treatment, its objectives, its theoretical advantages and disadvantages will be presented. The Informed Consent document is given to the patient and they are asked to review it, make notes and ask questions prior to agreeing to participate in this protocol. The patient is reassured that participation on this trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The physician assures the patient that if alternative therapy or no therapy at all is preferred, we will do all that we can to facilitate consultation with the appropriate referral organizations. The Informed Consent document may be obtained from the patient by the principal investigator, associate investigators, or the medical staff fellow under the supervision of the principal investigator.

Consent for the optional biopsies on this study will be obtained at the time of the procedure, using the procedure consent. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.6.1 Telephone re-consent procedure

Re-consent on this study may be obtained via telephone. Telephone consent will be obtained per OHSRP/IRBO and CCR policies and procedures.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.2 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI CCR

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research

objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 LMB-100 (IND # 152907)

14.1.1 Source

LMB-100 was transferred to the NIH CC Pharmacy by Roche, the drug manufacturer. For this trial, the drug will be supplied by the NIH CC Pharmacy.

14.1.2 Toxicity

Information in this section is based on preclinical studies with LMB-100, and clinical studies of the cytolytic fusion protein SS1-P. Patients should receive a full dose of LMB-100 unless a DLT and/or a treatment limiting toxicity is observed. In case of DLT and/or treatment limiting toxicities, treatment with LMB-100 will be stopped until resolution of toxicity to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities. A delay of LMB-100 administration for up to three weeks of the planned schedule will be acceptable to allow for resolution of toxicity to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities. If toxicity does not resolve to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities and the patient is unable to resume treatment with LMB-100 after this time, no additional doses will be administered and the patient will be withdrawn from study treatment.

14.1.2.1 Infusion-Related Reactions and Hypersensitivity Including Anaphylaxis

LMB-100 administration may cause infusion-associated symptoms such as fever, chills, hypotension, shortness of breath, skin rash, headache, nausea, and/or vomiting. Such reactions typically occur during or shortly after an infusion, predominantly the first infusion. Patients may also develop IgE-mediated hypersensitivity reactions to LMB-100. IRRs may be indistinguishable from an anaphylactic reaction. Patients should receive full supportive care to treat IRRs or anaphylaxis according to institutional practice. If infusion-associated signs or symptoms occur, patients should be monitored until complete resolution.

In vitro data suggest that the risk for the release of pro-inflammatory cytokines upon first administration of LMB-100 to humans is low (human whole blood assay, see section 1.2.3.3.3). Past experience with monoclonal antibodies that demonstrated a risk in the whole blood assay has shown that this risk could be effectively managed in the clinic with appropriate risk-minimization measures. The release of pro-inflammatory cytokines is believed to be partially responsible for the occurrence of IRRs.

14.1.2.2 Risk of Immunogenicity and Potential Safety Impact

LMB-100 may cause the formation of ADAs. These may trigger hypersensitivity reactions or immune complex-mediated responses. The development of ADAs to LMB-100, an improved cytolytic fusion protein with a humanized targeting moiety directed against mesothelin and a de-humanized, truncated Pseudomonas exotoxin A is expected to be less likely than SS1P. Clinical trials with SS1P have led to the development of neutralizing ADAs in 75% and 88% of patients after 1 cycle of therapy, in the IV bolus and continuous infusion trials respectively.[12]

Patients will be monitored at regular intervals for the development of ADAs and cytokines. In particular, any clinical signs and symptoms suggestive of a hypersensitivity reaction and/or an immune complex-mediated reaction possibly due to ADA formation will be carefully investigated.

14.1.2.3 Risk of Inflammatory Reactions to Serosal Membranes

LMB-100 administration may cause inflammatory reactions to serosal membranes including pleuritis, characterized by pleuritic chest pain, dyspnea, and hypoxia and pericarditis, characterized by precordial chest pain, congestive heart failure, hypotension, and uremia. Clinical trials with SS1P monotherapy have led to reversible pleuritis and pericarditis. Patients who develop symptoms of serosal inflammation should be closely monitored and receive standard treatments which may include corticosteroids.

14.1.2.4 Risk of Vascular Leak Syndrome

LMB-100 administration may cause VLS characterized by hypotension, hypoalbuminemia, edema, weight gain, and hemoconcentration. Clinical trials with SS1P monotherapy have led to the development of reversible VLS. Patients will be monitored with frequent assessments of chest x-rays, weight, edema, blood pressure, and serum albumin levels prior to and during treatment. Patients who develop symptoms of VLS should be closely monitored and receive standard symptomatic treatments.

14.1.2.5 Risk of Renal Toxicity

LMB-100 administration may cause renal toxicity characterized by increased creatinine, BUN, and proteinuria. In preclinical cynomolgus monkey studies, LMB-100 has shown increases in creatinine and histological changes including regenerative and degenerative changes to the tubular epithelium. Hemolytic uremic syndrome has been reported for other cytolytic fusion antibodies in development.

Patients should be monitored with renal laboratory assessments including creatinine, BUN, and urinalysis.

14.1.2.6 Injection Site Reactions

LMB-100 administration may cause adverse reactions at the infusion site characterized by pain, swelling, induration, and nodules. In preclinical NHP studies for both SS1P and LMB-100 reddening and swelling of the infusion site were noted. Patients who develop symptoms of infusion site reactions can be administered pain relieving medication (analgesic) as required, and rotation of infusion sites is recommended.

14.1.2.7 Pregnancy

No studies assessing the reproductive and developmental toxicity of LMB-100 have been conducted to date. It is not known whether LMB-100 can cross the placenta or cause harm to the fetus when administered to pregnant women or whether it affects reproductive capacity. LMB-100 should not be administered to pregnant women.

Use of effective contraceptive methods is recommended in all patients receiving LMB-100, including up to 4 months and 2 months following the last LMB-100 dose for women and men, respectively.

14.1.3 Formulation and preparation

LMB-100 drug product (20 mg/20 mL) is provided for syringe infusion as a sterile, colorless to brownish, preservative-free liquid in single-use, 20 mL vials. The nominal fill volume is 20 mL and the approximate concentration of LMB-100 recombinant fusion protein in the vials is 1 mg/mL.

14.1.4 Stability and Storage

Chemical and physical in-use stability for undiluted LMB-100 drug product in syringes has been demonstrated for 24 hours at 2-8 °C and 24 hours at ambient temperature.

Storage conditions should generally be at 2-8°C, but syringes may be held at room temperature for up to a maximum of 4 hours.

14.1.5 Administration procedures

Please refer to section 3.2.1

14.1.6 Incompatibilities

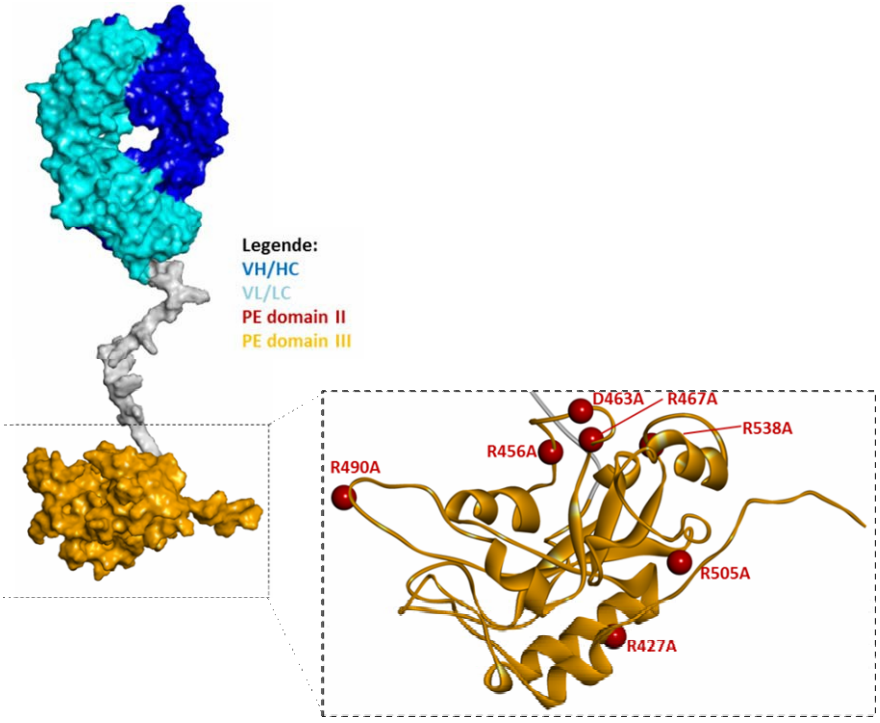
Pharmacodynamic drug interaction studies have not been conducted. LMB-100 is contraindicated in subjects with a history of severe allergic anaphylactic reactions to humanized, chimeric or mouse peptides/antibodies or to any components of the product.

14.1.6.1 Mechanism of action

LMB-100 is a novel recombinant anti-mesothelin targeted cytolytic fusion protein (cFP) developed for the treatment of patients with solid tumors that express the mesothelin protein. Mesothelin is a suitable candidate for targeted therapy due to its very limited expression in normal/non-malignant tissue and its high expression in several tumor entities including mesothelioma, ovarian cancer, pancreatic cancer, gastric cancer, breast cancer, and lung cancer. To target mesothelin, a humanized Fab fragment of the anti-mesothelin antibody SS1 is linked to a truncated and de-immunized recombinant 24 kD fragment of Pseudomonas exotoxin (PE24). After binding to mesothelin, the complex is internalized by endocytosis and kills cells by inhibition of eukaryotic elongation factor 2 (eEF2), leading to arrest of protein synthesis and secondarily triggering cell death by apoptosis or necrosis.

14.1.6.2 Molecular Weight: approximately 73 kDa

14.1.6.3 Chemical Structure



H1L1 polypeptide structure consisting of one variable heavy chain containing the Pseudomonas Exotoxin A moiety and one variable light change held together by a disulfide bond.

14.2 *NAB*-PACLITAXEL

Please refer to package insert for additional information.

14.2.1 Source

As of Amendment E, the study drug *nab*-paclitaxel will be provided under a Clinical Trial Agreement with the manufacturer, Celgene.. Commercially purchased *nab*-paclitaxel may be used until the Celgene supply is in place.

14.2.2 Toxicity

The most common clinically significant adverse reactions reported with *nab*-paclitaxel in patients with pancreatic cancer include: neutropenia, anemia, thrombocytopenia, pancytopenia peripheral neuropathy, cardiac failure, tachycardia, dyspnea, pneumonitis, nausea, vomiting, diarrhea abdominal pain, intestinal obstruction, colitisarthralgia/myalgia, renal failure, increased alanine aminotransferase, increased aspartate aminotransferase, increased bilirubin, and increased creatinine.

Hypersensitivity reactions including fatal anaphylactic reactions have rarely been reported with *nab*-paclitaxel. If a hypersensitivity reaction occurs, *nab*-paclitaxel should be discontinued immediately, symptomatic treatment should be initiated, and the participant should not be rechallenged with paclitaxel.

Bone marrow suppression (primarily neutropenia) occurs frequently with *nab*-paclitaxel. Frequent monitoring of blood cell counts should be performed. Participants should not be retreated with subsequent cycles of *nab*-paclitaxel until neutrophils recover to > 1500 cells/mm^2 and platelets to > 100,000 cells.

Sensory neuropathy occurs frequently with *nab*-paclitaxel, though generally not severe. The occurrence of Grade 1 or 2 sensory neuropathy does not generally require dose reduction.

Sepsis was reported in 5% of participants with or without neutropenia who received *nab*-paclitaxel.[40] Complications due to underlying pancreatic cancer, especially biliary obstruction or presence of biliary stent, were identified as significant contributing factors.

Pneumonitis occurred in 4% of participants using *nab*-paclitaxel. Closely monitor all participants for signs and symptoms of pneumonitis. After ruling out infectious etiology and upon making a diagnosis of pneumonitis, permanently discontinue treatment with *nab*-paclitaxel and promptly initiate appropriate treatment with supportive measures.

Hepatic impairment may increase the toxicity of *nab*-paclitaxel, particularly myelosuppression. Administration of *nab*-paclitaxel in participants with hepatic impairment should be performed with caution.

Congestive heart failure and left ventricular dysfunction have rarely been observed in participants receiving *nab*-paclitaxel. Most of these participants were previously exposed to cardiotoxic medicinal products such as anthracyclines or had underlying cardiac history. Participants receiving *nab*-paclitaxel should be vigilantly monitored for the occurrence of cardiac events.

Nausea, vomiting, and diarrhea following administration of *nab*-paclitaxel may be treated with commonly used anti-emetics and constipating agents.

In the very elderly (≥ 75 years) who received *nab*-paclitaxel, there was a higher incidence of serious adverse reactions and adverse reactions that led to discontinuation including hematologic toxicities, peripheral neuropathy, decreased appetite, and dehydration. Participants 75 years or older should be carefully assessed for their ability to tolerate *nab*-paclitaxel with special consideration to performance status, co-morbidities, and increased risk of infections.

14.2.3 Formulation and preparation

Formulation

ABRAXANE is supplied as a lyophilized powder containing 100 mg of paclitaxel formulated as albumin-bound particles in single-use vial for reconstitution.

Preparation

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the INSIDE WALL OF THE VIAL.

3. DO NOT INJECT the 0.9% Sodium Chloride Injection, USP, directly onto the lyophilized cake as this will result in foaming.
4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilized cake/powder.
5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.
6. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.

Calculate the exact total dosing volume of 5 mg/mL suspension required for the participant: Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

The reconstituted suspension should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be gently inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion.

Inject the appropriate amount of reconstituted ABRAXANE® into an empty, sterile IV bag (plasticized polyvinyl chloride (PVC) containers, PVC or non-PVC type IV bag). The use of specialized DEHP-free solution containers or administration sets is not necessary to prepare or administer ABRAXANE infusions. The use of an in-line filter is not recommended.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

14.2.4 Stability and Storage

Unopened vials of ABRAXANE are stable until the date indicated on the package when stored between 20°C to 25°C (68°F to 77°F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Product should be retained in the original packaging to protect from bright light.

Stability of Reconstituted Suspension in the Vial

Reconstituted ABRAXANE should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) for a maximum of 24 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag

The suspension for infusion when prepared as recommended in an infusion bag should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) and protected from bright light for a maximum of 24 hours..

14.2.5 Administration procedures

Please see section 3.2.2.

14.2.6 Incompatibilities

Please refer to the package insert.

15 REFERENCES

1. Sugarbaker DJ, Garcia JP, Richards WG, Harpole DH, Jr., Healy-Baldini E, DeCamp MM, Jr., Mentzer SJ, Liptay MJ, Strauss GM, Swanson SJ: **Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma. Results in 120 consecutive patients**. *Annals of surgery* 1996, **224**(3):288-294; discussion 294-286.

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16 APPENDICES

16.1 APPENDIX A – PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 APPENDIX B – COCKCROFT-GAULT FORMULA FOR CALCULATION OF CREATININE CLEARANCE

A commonly used surrogate marker for actual creatinine clearance is the Cockroft-Gault formula, which employs creatinine measurements and a patient's weight to predict the clearance. The formula, as originally published, is:

$$x = \frac{(140 - age) \times weight}{72 \times creatinine}$$

This formula expects weight (actually mass) to be measured in kilograms and creatinine to be measured in mg/dL, as is standard in the USA. The resulting value is multiplied by a constant of 0.85 if the patient is female. This formula is useful because the calculations are relatively simple and can often be performed without the aid of a calculator.

A modification of this formula, useful for the common units of measure, is:

$$x = \frac{(140 - age) \times weight \times constant}{creatinine}$$

This formula uses metric units (weight in kilograms, creatinine in µmol/L). The constant is 1.23 for men and 1.04 for women.