Abbreviated Title: Local LMB-100 + ipilimumab

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Title: Phase I Study of Intratumor Injection of anti-Mesothelin Immunotoxin LMB-100 with Ipilimumab in Malignant Mesothelioma

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Drug Name:	LMB-100	Ipilimumab (commercial)
IND Number:	152907	152907
Sponsor:	Center for Cancer Research	Center for Cancer Research
Manufacturer:	Selecta Biosciences	Bristol-Myers Squibb
Supplier	CC Pharmacy	CC Pharmacy

PRÉCIS

Background:

- LMB-100, and a closely related immunotoxin, SS1P, also targeting mesothelin, given intravenously, have been studied in Phase 1 clinical studies for mesothelioma and pancreatic cancer.
- LMB-100 given intravenously, results in systemic inflammation in patients, but as a single agent has limited anti-tumor efficacy.
- Almost all patients develop neutralizing anti-LMB-100 antibodies after 2 cycles of therapy.
- Intra-tumoral delivery of LMB-100 has been shown to induce immune cell infiltration in immune-competent mice, bearing murine malignant mesothelioma tumors. Combination with CTLA-4 blockage eradicates murine tumors by promoting anti-cancer immunity.
- Ipilimumab is a fully human anti CTLA-4 monoclonal antibody, that is approved for treatment of melanoma and in combination with nivolumab for many solid tumors.
- It is hypothesized that intra-tumoral delivery of anti-mesothelin immunotoxin LMB-100 in combination with ipilimumab will result in greater anti-tumor efficacy in patients with mesothelioma.

Objective:

- To determine the safety and feasibility of intra-tumoral LMB-100 injection plus ipilimumab infusion in patients with mesothelioma
- To identify the recommended phase 2 dose (RP2D) of intratumorally administered LMB-100 + ipilimumab in patients with malignant mesothelioma

Eligibility:

- Histologically confirmed pleural or peritoneal mesothelioma not amenable to potentially curative surgical resection.
- Have locally accessible disease suitable for intra-tumor injection of LMB-100. This includes superficial or visceral lesions.
- Subjects must have received prior immune checkpoint therapy with anti-PD-1/PD-L1 inhibitors alone or in combination with anti-CTLA4 blocking antibodies, as well as platinum-based chemotherapy.
- Age ≥ 18 years.
- ECOG performance status of 0 or 1.
- Adequate organ and bone marrow function
- Subjects with clinically significant pericardial effusion are excluded
- Chemotherapy within 3 weeks or radiotherapy within 2 weeks prior to start of study therapy is prohibited.
- Subjects with active CNS metastasis are excluded

- Subjects with active autoimmune disease for which they have received systemic immunosuppressive medications during the previous 2 years (excluding daily glucocorticoid-replacement therapy for conditions such as adrenal or pituitary insufficiency) are excluded
- Subjects with active interstitial lung disease, or a history of pneumonitis or interstitial lung disease for which they had received glucocorticoids are excluded

Design:

- This is an open-label, single center phase I dose escalation study of intratumorally administered LMB-100 followed by ipilimumab in subjects with malignant mesothelioma.
- Subjects will receive intratumorally administered LMB-100, beginning at dose level 1, in 21-day cycles. LMB-100 will be given on days 1 and 4 of cycle 1, and ipilimumab is given on day 1 of cycles 2 – 4.
- Tumor biopsies will be performed prior to each administration of LMB-100,on day 1 of cycle 2 and after completion of ipilimumab therapy to evaluate changes in the tumor immune microenvironment
- Up to 14 evaluable subjects will be enrolled.

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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 Objectives

- To determine the safety and feasibility of intra-tumoral LMB-100 injection plus ipilimumab infusion in patients with mesothelioma.
- To identify the recommended phase 2 dose (RP2D) of intratumorally administered LMB-100 + ipilimumab in patients with malignant mesothelioma.

1.1.2 Secondary Objectives

- To preliminarily determine the objective response rate of combination therapy with intratumorally administered LMB-100 plus ipilimumab.
- To determine the duration of response, progression free survival and overall survival with intratumorally administered LMB-100 plus ipilimumab.

1.1.3 Exploratory Objectives

- To assess the effect of ipilimumab on pharmacokinetics and antidrug antibody development against LMB-100
- To establish the correlation of response with tumor mesothelin expression.
- To evaluate changes in the tumor microenvironment following treatment with intratumorally administered LMB-100 and ipilimumab.
- To evaluate the utility of serum mesothelin and megakaryocyte potentiating factor (MPF) as a biomarkers of tumor response.
- To evaluate proinflammatory cytokine release in response to LMB-100 administration

1.2 BACKGROUND AND RATIONALE

1.2.1 Hypotheses:

- Treatment with intratumorally administered LMB-100 and combination ipilimumab will induce potent immune mediated anti-tumor response in treatment refractory mesothelioma
- LMB-100 will induce tumor inflammation that leads to recruitment of CD8+ cells in the tumor and administration of ipilimumab will increase the efficacy of these cytotoxic T cells.

1.2.2 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 form of mesothelioma and highly expresses mesothelin. 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]. For patients who progress on this regimen, systemic chemotherapy options are limited in number and efficacy.

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.

1.2.3 Background on the Intra-tumoral Administration of Immunotherapy

Immune escape is commonly employed by tumors to create a more immunosuppressive tumor microenvironment. This frequently occurs through a variety of methods, including immune editing, downregulation of tumor associated antigens, changes in T-cell subpopulation, and secretion of immunosuppressive cytokines and immunosuppressive checkpoint proteins[8-10]. Tumor draining lymph nodes are believed to be central to controlling the net immune response to malignancies, via balancing anti-tumor responses with immune tolerance[9, 10]. The majority of interactions that occur in these lymph nodes, are primarily driven by cellular interactions at the local level, involving antigen presenting cells and effector cells[11]. By modulating immune activity at a local level, locally administered therapies have the potential to enhance the anti-tumor immune response, both locally and systemically. Additionally locally administered therapies have the potential for increased immunogenic cell death, via increased bioavailability making it possible to avoid toxicities associated with higher doses required with systemic administration[12]. More recently the potential of local administered immunotherapies has proven successful in melanoma, with Talimogene Laherparepvec being approved for locally advanced melanoma post-surgery[13, 14].

1.2.4 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, peritoneum and pericardium[15]. 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)[16]. For NSCLC, mesothelin expression has been found primarily in lung adenocarcinomas, with expression ranging from 53%-70%, with expression associated with a poor clinical prognosis, often being associated with *KRAS* mutations. Moreover for advanced lung adenocarcinoma, high expression of mesothelin (>25% of mesothelin positive cells) has been seen in 24% of Stage III and IV patients[17]. 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[18].

Because of the high expression of mesothelin in many malignancies, a variety of agents are being developed to target mesothelin including use of anti-mesothelin drug conjugates, vaccines and CAR-T cells. 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. A variety of treatments directed against mesothelin, including antibody-based therapies, mesothelin vaccines, and adoptive T-cells, are in the process of being investigated[19]. The Laboratory of Molecular Biology (LMB) and the Thoracic and GI Malignancies Branch (TGMB), Center for Cancer Research, National Cancer Institute have pioneered the use of mesothelin- targeted agents and clinical trials over the last decade.

1.2.5 LMB-100:

LMB-100 is a recombinant immunotoxin consisting of a humanized anti-mesothelin Fab fused to a 24kDa truncated *Pseudomonas* exotoxin A (PE) fragment, with mutations that suppress B and T cell epitopes[20]. After entering the cell by endocytosis, the immunotoxin reaches the cytosol, where it inactivates elongation factor 2, arrests protein synthesis and induces apoptotic cell death. LMB-100 has anti-tumor efficacy against several mesothelin expressing tumor models including mesothelioma PDX models[21]. A phase I clinical trial of LMB-100 has been completed at NCI and we have established its MTD when given intravenously.

1.2.5.1 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 1 for structure) is a next generation PE-fusion protein that has been protein-engineered to reduce its immunogenicity by:

1. Using a fully humanized Fab fragment derived from the anti-mesothelin antibody SS1 for tumor targeting
2. 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
3. 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[22], but has also resulted in reduced incidents of CLS in animal models of CLS[23].

1.2.5.2 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 1). 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[23, 24]. 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.

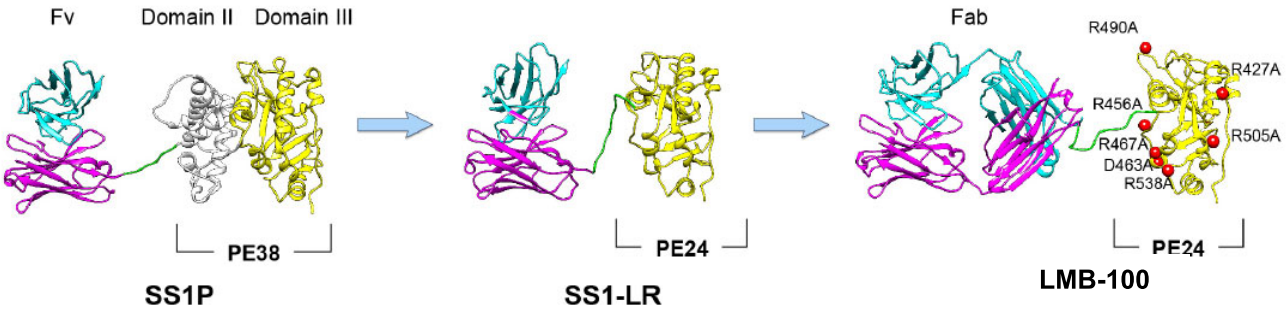


Figure 1. Structural models of SS1P and its de-immunized variants SS1P-LR and LMB-100 are shown.

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.

1.2.5.3 Nonclinical Studies

1.2.5.3.1 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[20, 25].

Animal studies demonstrated that a single cycle of LMB-100 treatment given at an optimal dose of approximately 2 mg/kg, 3 x 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.

1.2.5.3.2 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 x 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.

1.2.5.3.3 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 x 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 mcg·h/mL (study day 1, preliminary data). In a subsequent 1 cycle GLP study (QOD x 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 mcg·h/mL after the first and third dose (preliminary data).

The potential of LMB-100 to induce off-target capillary 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 CLS. 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.

1.2.5.4 Clinical testing of LMB-100

The first in human study of LMB-100 was initiated by Roche (NCT02317419) and later on continued and completed at NCI (NCT02798536). Twenty-five patients received at least one infusion of LMB-100 and were evaluable for safety assessment (Table 1). The median age of patients was 62.5 years and 14 were females. Seventeen patients had mesothelioma, 2 gastric cancer and 3 each ovarian and pancreatic cancer. Fifteen patients were treated on the multicenter Phase I conducted by Roche, and 10 on the NCI Phase I part of the study.

Table 1. Patient Demographics and Clinical Characteristics

Characteristics	Patients (n = 25)
Sex	
Male	11
Female	14
Age (years)	
Median (range)	62.5 (37-79)
ECOG Performance Status	
0	9
1	16
Diagnosis	
Malignant Mesothelioma	17
Gastric Cancer	2
Ovarian Cancer	3
Pancreatic Cancer	3
Phase I cohorts	
Multicenter Phase I	15
NCI Phase I	10
LMB-100 Treatment Cycles	
Median (range)	2 (1-7)

Seven different dose levels of LMB-100 were evaluated ranging from 45 to 250 mcg/kg (Table 2). On the multi-center part of the phase I study doses of 45, 65, 100, and 170 mcg/kg were evaluated before DLT (capillary leak syndrome, CLS) was observed in 2 of 4 patients treated at 250 mcg/kg. Subsequently, the dose was de-escalated, and 2 additional participants were enrolled at a 200 mcg/kg dose level. However, these two patients were not evaluable for DLT evaluation since they received only a single infusion of LMB-100 before the study was terminated. For the NCI part of the phase I study, treatment began at 170 mcg/kg and 3 of 3 patients experienced creatinine increase (grade 2) during the first cycle. This led to a delay in cycle 2 treatment, an investigator-defined DLT. Kidney function of all 3 participants recovered to baseline and they were able to

receive Cycle 2 of therapy at 140 mcg/kg without any DLT. Seven participants were then treated at reduced dose level of 140 mcg/kg, which was found to be the MTD. Asymptomatic, grade 3 hyponatremia in the setting of CLS was noted in 1 patient at the 140mcg/kg dose level which was a DLT.

Table 2: Dose escalation scheme of LMB-100 and DLTs

Dose Level	LMB-100 Dose (mcg/kg)	No. of patients treated	Patients with DLT*	DLT*
Multicenter Phase I				
1	45	1	0	-
2	65	1	0	-
3	100	3	0	-
4	170	4	0	-
5	200	2	NE	-
6	250	4	2	CLS
NCI Phase I				
4‡	170	3	3	Creatinine Increase
7	140	7	1	Hyponatremia

*DLT, dose-limiting toxicity; NE, not evaluable; CLS, capillary leak syndrome; ‡received cycle 1 at dose level 4 and subsequent cycles at dose level 7.

1.2.5.4.1 Safety

All adverse events possibly, probably or definitely related to LMB-100 treatment are shown in Figure 2. The most common toxicities seen across all dose levels included hypoalbuminemia (80%), myalgia (60%), peripheral edema (52%), weight gain (36%) and creatinine increase (32%) as manifestation of CLS (Figure 2A). Other frequent toxicities were nausea (52%), fatigue (52%), dyspnea (36%) and infusion related reactions (IRR) (32%). Most of the toxicities were grade 1 and 2. Grade 3 toxicities included dyspnea (1 patient), IRR (1 patient), anemia (1 patient), hyponatremia (2 patients), decreased lymphocyte count (1 patient) and arthritis (1 patient). The adverse events observed in the seven patients treated at the LMB-100 MTD of 140 mcg/kg are shown in Figure 2B. One patient had grade 3 hyponatremia which was considered as DLT. Based on the high incidence of IRR we instituted routine prophylaxis with acetaminophen, diphenhydramine and famotidine before each dose of LMB-100 for all patients treated at NCI.

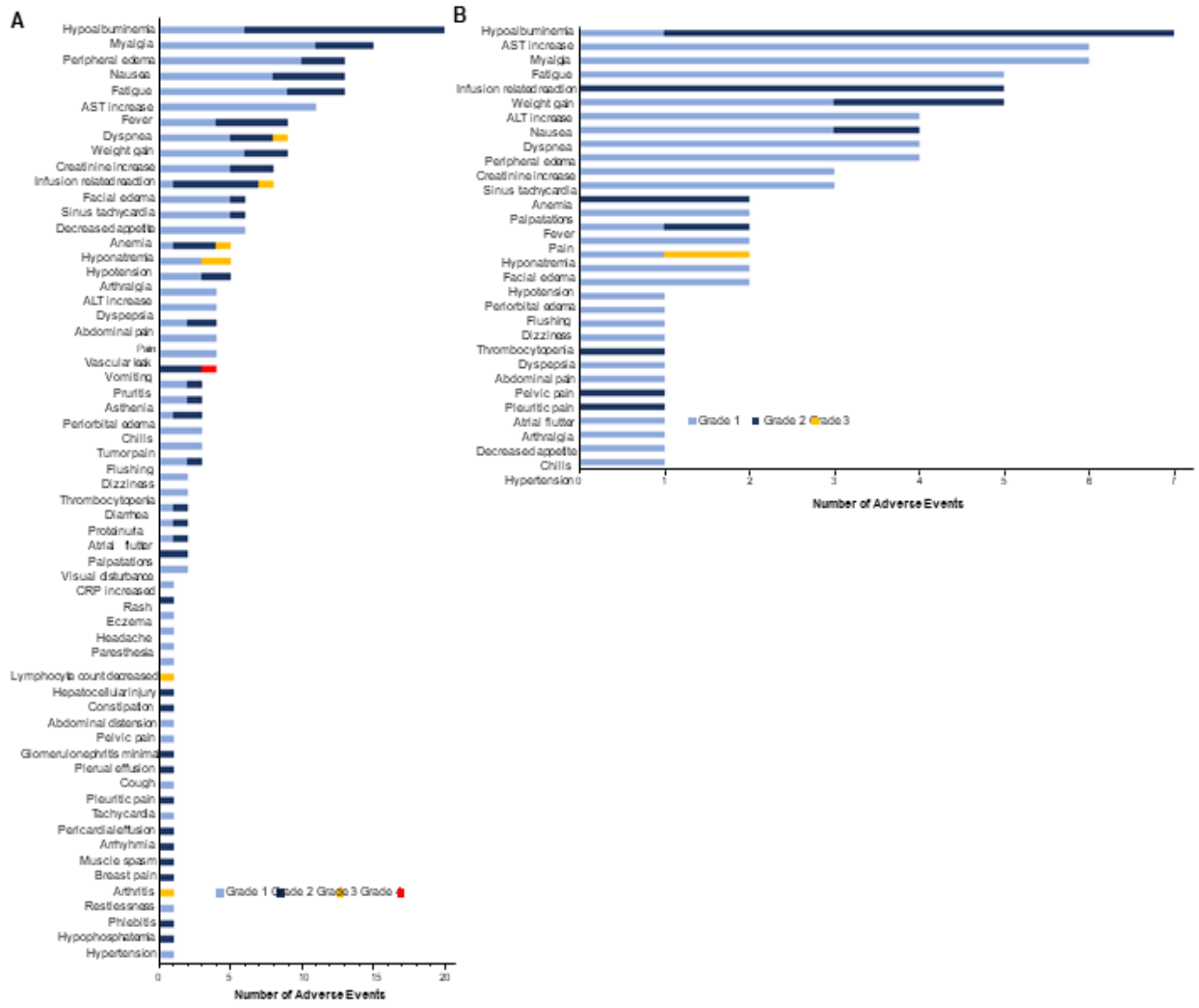


Figure 2:LMB-100 related adverse events (AEs) that were possibly, probably or definitively related to LMB-100 are shown (**A)** AE seen at all doses of LMB-100 evaluated. (**B)** AEs seen at the MTD of LMB-100.

1.2.5.4.1.1 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.

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 1.2.5.3.3). 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.

1.2.5.4.2 Pharmacokinetics of LMB-100

The change in LMB-100 exposure over time at the different dose levels during cycle 1 day 1 infusion is shown in Figure 3A. There was a dose-proportional increase in both the C_{max} and AUC_{INF} from 45 mcg/kg to 250 mcg/kg (Figure 3B and C). In patients who were treated at the LMB-100 MTD of 140 mcg/kg, the mean C_{max} was 1974 ng/ml, mean AUC_{INF} 3709, mean half-life 0.87 hour (53 minutes), mean clearance 3.98 L/hour and the mean volume of distribution was 5.90 L. The C_{max} of LMB-100 after day 1 infusion of LMB-100 during cycles 1, 2, 3 and 4 are shown in Figure 3D. In cycle 1 all patients had C_{max} >100 ng/mL; in cycle 2, 13 of 21 (62%) patients who received LMB-100 had C_{max} >100 ng/mL; during Cycle 3 this was reduced to 2 of 11 (18%) patients. All 9 patients who received Cycle 4 had undetectable plasma drug concentration after LMB-100 infusion (Figure 3D).

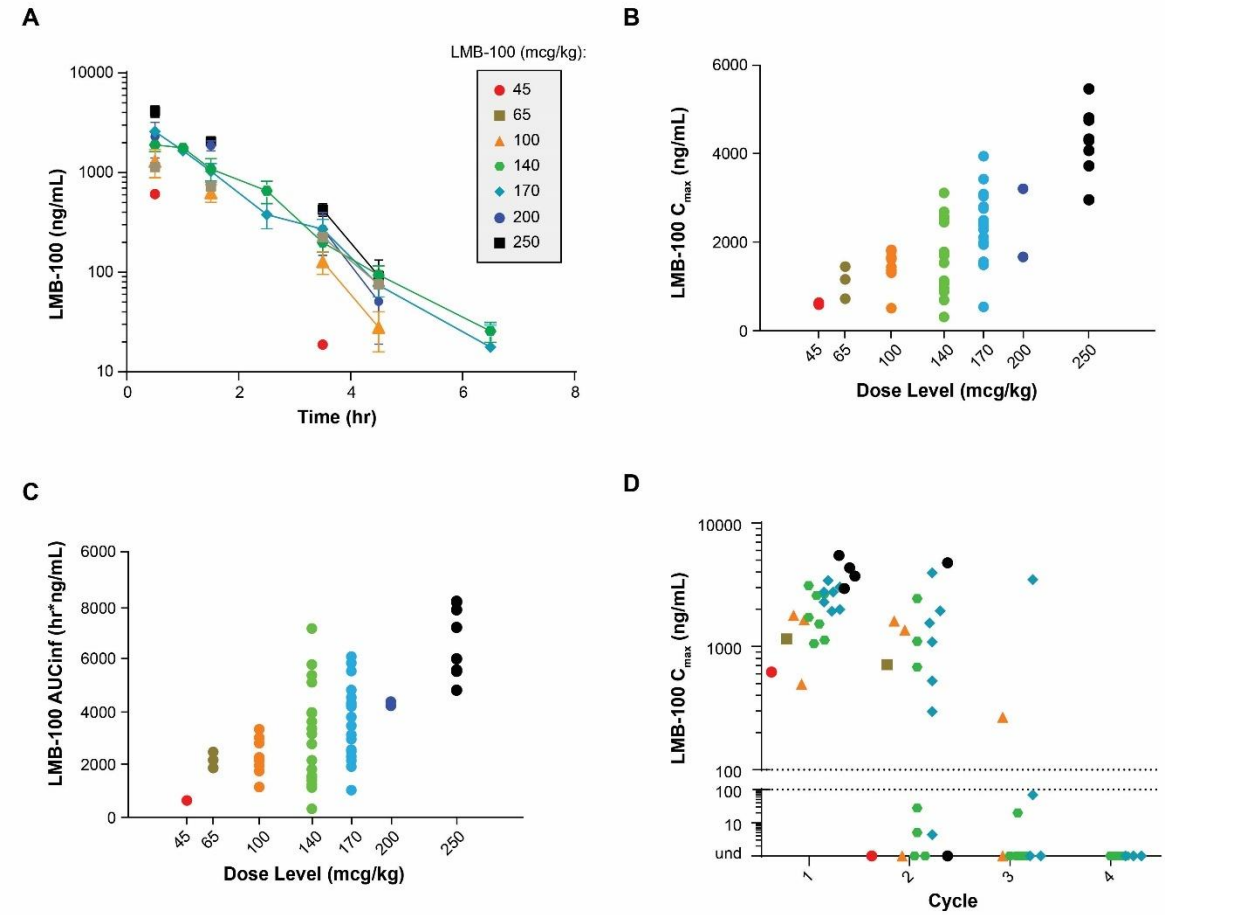


Figure 3: Pharmacokinetics of LMB-100. **(A)** Change in LMB-100 exposure over time after first infusion. (**B**) Dose-proportional relationship of C_{max} and (**C**) AUC_{INF} versus dose level. (**D**) Change in C_{max} by cycle of LMB-100 administered.

1.2.5.4.3 Human anti-LMB-100 antibodies

The presence of ADA was assessed retrospectively, and this information was not available to guide decisions concerning administration of LMB-100. Presence of ADA, of any titer, at baseline i.e. prior to receiving first cycle of LMB-100 did not affect the LMB-100 blood levels during cycle 1, since all 23 patients had LMB-100 C_{max} levels ≥ 495 ng/ml on the day 1 infusion. During cycle 2, 13 of 21 patients who received LMB-100 achieved good blood levels (arbitrarily defined as levels

>100 ng/ml; in-vitro most mesothelin expressing patient derived mesothelioma cell lines are sensitive to LMB-100 at concentrations of <10 ng/ml) [21], and this was associated with low-titer ADA.

1.2.5.4.4 Efficacy

Of the 25 patients enrolled on the study 20 were evaluable for response. Response data were unavailable for the 5 patients (2 patients who received single dose of LMB-100 at 200 mcg/kg dose level and 3 patients treated at the 250 mcg/kg dose level) on the multi-center phase I study who were taken off treatment due to study discontinuation. Of the 20 patients evaluable for tumor response, 10 had stable disease and 10 had progressive disease as their best response. There were no objective radiologic partial or complete responses.

The characteristics of the 10 mesothelioma patients treated on the NCI phase I study are shown in Table 3. Patients P01, P02 and P03 received LMB-100 at 170 mcg/kg during cycle 1 and 140 mcg/kg during subsequent cycles, whereas patients P04 to P10 received 140 mcg/kg of LMB-100 during all cycles of treatment. Three patients had pleural mesothelioma and 7 had peritoneal mesothelioma. Tumors from all these patients had high mesothelin expression with at least ≥50% tumor cells expressing it with a 2-3+ staining intensity. Eight patients had stable disease and 2 had progressive disease. The median PFS was 2.8 months (95% CI: 1.5 -3.0 months) with all patients progressing within approximately 3 months of starting LMB-100 treatment; median overall survival was 23.4 months (95% CI: 6.8 – 34.6 months). Four patients had survival of greater than 30 months and out of these, 2 patients are alive at last follow-up (November 1, 2019). Five of 10 patients had a decrease in serum mesothelin levels after 2 cycles of LMB-100 as compared to pre-treatment serum mesothelin. However, given the small number of patients treated, it is difficult to draw any conclusions correlating stable disease with changes in serum mesothelin levels.

Table 3: Clinical characteristics and treatment response in mesothelioma patients treated at the 170 and 140 mcg/kg dose levels.

Pt. ID	Age/ Sex	Site of disease	Histologic Subtype	Tumor MSLN¶	Radiologic Response*	PFS (months)	OS (months)	% Change in serum MSLN (Post C2)	% Change in serum MPF (Post C2)#
^P01	37/ F	Peritoneal	Epitheliod	2-3+ >90%	SD post C4	2.7	34.6	16.6	70.22
^P02	72/ M	Peritoneal	Epitheliod	3+ 100%	SD post C4	3.0	27.7	15.2	-31.43
^P03	66/ M	Pleural	Epitheliod	3+ 90%	SD post C2	2.7	33.5	3.3	23.42
P04	67/ F	Peritoneal	NS	90%	SD after C2	2.9	9.4	-27.0	-42.57
P05	68/ M	Pleural	Epitheliod	3+ 95%	PD post C2	1.5	37.0	70.1	104.67
P06	46/ F	Peritoneal	Epitheliod	3+ >50%	SD post C4	3.1	16.9	6.1	96.94
P07	48/ F	Peritoneal	Epitheliod	3+ 100%	SD post C4	2.7	36.8	-12.9	-1.04
P08	68/ M	Pleural	Epitheliod	3+ 50%	PD post C4	3.2	6.8	-59.4	141.38
P09	40/ F	Peritoneal	Epitheliod	3+ 100%	SD post C3	2.7	11.3	-7.5	-11.34

Pt. ID	Age/ Sex	Site of disease	Histologic Subtype	Tumor MSLN¶	Radiologic Response*	PFS (months)	OS (months)	% Change in serum MSLN (Post C2)	% Change in serum MPF (Post C2)#
P10	59 /F	Peritoneal	NS	3+ 100%	SD post C4	2.8	19.1	-31.8	107.32

MSLN, Mesothelin; NS, Histologic subtype of mesothelioma not specified in the pathologist's report; SD, Stable Disease; PD, Progression of Disease; PFS, Progression Free
survival; OS, Overall Survival; C, Cycle
^Patients received LMB-100 170 mcg/kg during cycle 1 and 140 mcg/kg during subsequent cycles.
¶ MSLN expression determined by IHC
* Best response at completion of LMB-100 treatment
+ alive
#Change in serum mesothelin at end of two cycles of LMB-100

1.2.5.4.4. LMB-100 causes systemic inflammation and immune cell infiltration in tumor

Amongst the patients treated on the Phase I trial of LMB-100 who had been subsequently challenged with anti-PD1 antibodies , durable responses of >12 months were seen in 4 of 7 patients, including one with on-going CR at 36 months[26]. To understand whether treatment with LMB-100 could make patient tumors more sensitive to anti PD-1 antibody, pembrolizumab, we studied the ability of LMB-100 to induce a systemic inflammatory response and its effect on immune cell populations within the tumor. To do so, we analyzed the C-reactive protein (CRP) concentrations, the cytokine profile, and gene expression in tumor biopsies of patients who were treated on the phase I clinical trial with LMB-100 alone. As shown in Fig. 4A, the concentration of CRP, an acute phase reactant, in the serum increased substantially in 6 of 7 patients on cycle 1 day 5, after two doses of LMB-100 (p=0.031). After completion of LMB-100 therapy, there was a gradual decrease in CRP to baseline values, and a representative example is shown in Fig. 4B. Next, we analyzed the cytokine profile of the 10 patients before and after LMB-100 treatment. IFN-γ concentrations increased within 6 hours of LMB-100 treatment in 5 of 8 patients with detectable baseline values. In addition, proinflammatory cytokines IL-8, IL-6, and MCP-1 were increased in 9 of 10 patients, and IL-18 was increased in 7 of 10 patients on day 5, after two doses of LMB-100 treatment on days 1 and 3 (Fig. 4C). Taken together, these results show that treatment with LMB-100 results in a systemic inflammatory response. NanoString nCounter Gene Expression Assay was used to detect cancer immune-related gene expression in pre- and post- treatment tumor biopsies of these six patients treated with LMB-100. Because lymphocyte infiltration is a key factor for effective anti-PD-1 and anti-PD-L1 therapy[27], we evaluated different cell type signatures using NanoString advanced analysis[28], which included CD45, Th1 cells, CD8+ T cells, exhausted CD8+ cells, DCs, and macrophages. Treatment with LMB-100 increased scores of CD45, $CD8^+$ T cells, exhausted $CD8^+$, DCs, and macrophages in 4 of 6 patients, and Th1 cell score in 3 of 6 patients (Figure 4D).

These results indicate that LMB-100 causes systemic inflammation and increase immune cell infiltration in the tumor. This provides a rationale that intra tumoral injection of LMB-100 can provide much greater inflammation and immunogenic cell death.

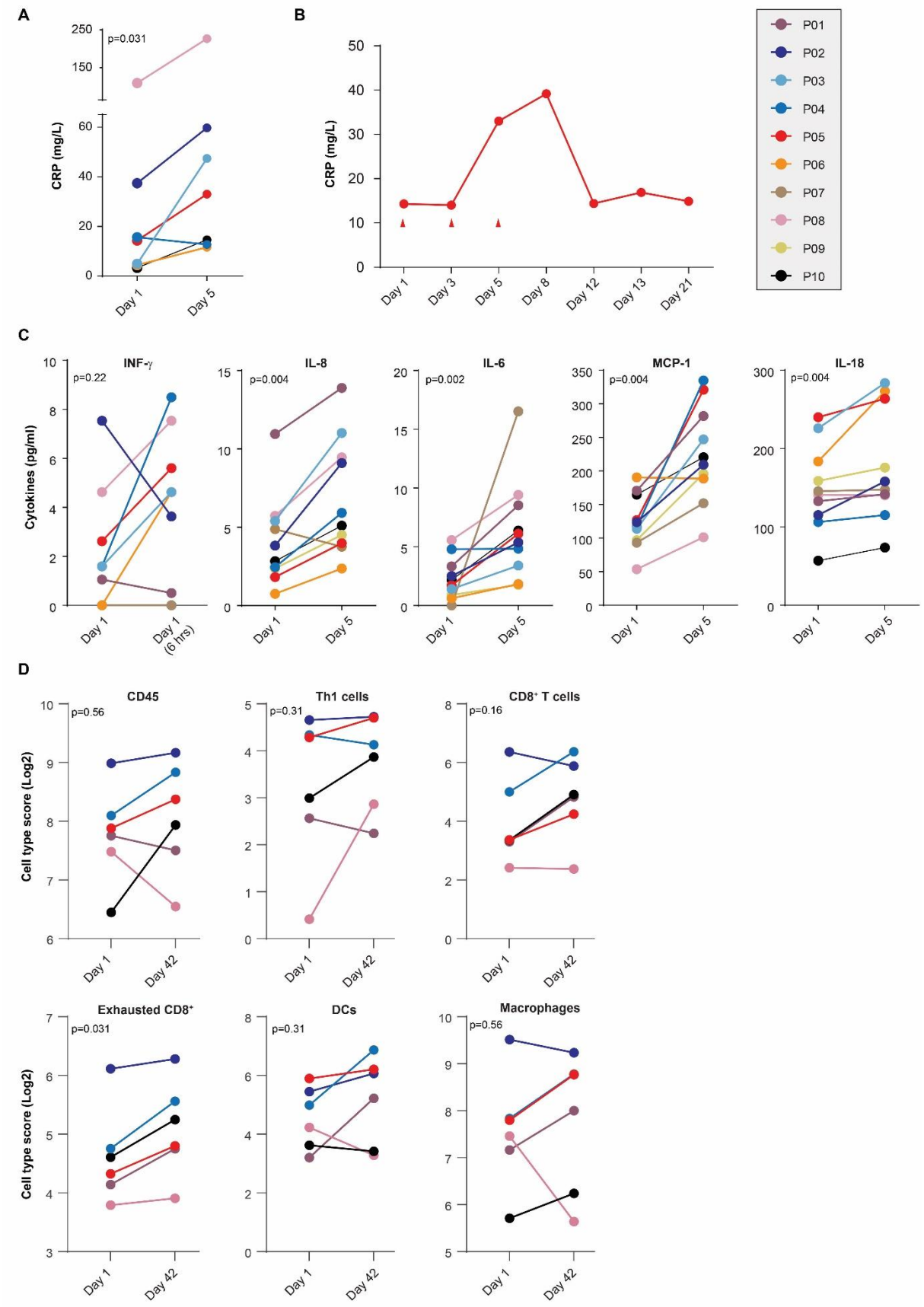

A
p=0.031
CRP (mg/L)
Day 1
Day 5
B
CRP (mg/L)
Day 1
Day 3
Day 5
Day 8
Day 12
Day 13
Day 21
P01
P02
P03
P04
P05
P06
P07
P08
P09
P10
C
INF-γ
p=0.22
IL-8
p=0.004
IL-6
p=0.002
MCP-1
p=0.004
IL-18
p=0.004
Cytokines (pg/ml)
Day 1
Day 1 (6 hrs)
Day 5
D
CD45
p=0.56
Th1 cells
p=0.31
CD8⁺ T cells
p=0.16
Exhausted CD8⁺
p=0.031
DCs
p=0.31
Macrophages
p=0.56
Cell type score (Log2)
Day 1
Day 42

Figure 4. LMB-100 alone induced immune responses in mesothelioma patients. (**A**) CRP concentrations in the serum of mesothelioma patients on Days 1 and 5 of Cycle 1 of LMB-100 treatment. (**B**) Change in CRP concentration in patient P05 in response to LMB-100 through cycle 1. Red arrowheads indicate days when LMB-100 was administered. (**C**) Cytokine concentrations in plasma from patients with mesothelioma on Days 1 and 5 of LMB-100 cycle 1. IFN-γ was evaluated before and 6 hours after LMB-100 treatment on Day 1. (**D**) Cancer immune gene expression analyzed by NanoString in tumor biopsies of patients obtained before (day 1) and after (day 42) LMB-100 treatment. Different cell type gene expression signature sets were scored using NanoString advanced analysis modules. Cell type scores are shown as log2 scale.

1.2.5.4.5 Summary

The phase I clinical trial has established tolerability, MTD, immunogenicity and pharmacokinetics of LMB-100. The MTD of LMB-100 was 140 mcg/kg and the DLT was CLS. All patients including those with pre-existing ADA at baseline had good blood levels during cycle 1 and 60% of patients had good LMB-100 blood levels during cycle 2. Development of ADA prevented good LMB-100 levels during cycles 3 and 4. Of the 20 patients evaluable for response, 10 had stable disease as best response and 10 had progressive disease. Of the 10 mesothelioma patients treated at MTD, 8 had stable disease and 2 had PD. There were no objective radiologic responses.

Although LMB-100 by itself has limited clinical activity, preclinical studies support its development as part of combination studies which are currently in the clinic. The two main approaches being explored include, studies in combination with immune checkpoint inhibitors and studies to decrease antibody formation to LMB-100. Preclinical studies of LMB-100 show remarkable synergistic anti-tumor efficacy with anti-CTLA4 as well as anti-PD-1 antibodies using immunocompetent mouse models[29]. Additionally, amongst the patients who received anti-PD1 antibodies after LMB-100, durable responses of >12 months were seen in 4 of 7 evaluable patients, including one with on-going complete response at 36 months[26]. While LMB-100 causes cytotoxicity, it has also been observed to induce immune activation, leading to immunogenic cell death[30]. This has led to two Phase II clinical trials testing sequential LMB-100 followed by pembrolizumab in patients with refractory mesothelioma (NCT03644550) and non-small cell lung cancer (NCT04027946). The patients are treated with 2 cycles of LMB-100 (140 mcg/kg QOD x 3 doses every 3 weeks), followed by pembrolizumab 200 mg every 3 weeks until disease progression, unacceptable toxicity or maximum of 2 years.

1.2.6 Ipilimumab

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4, Yervoy™) is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells.[31] CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and inhibits its interaction with ligands on antigen-presenting cells (APCs). The proposed mechanism of action for ipilimumab in subjects with melanoma is indirect, possibly through T-cell potentiation and mediation of antitumor immune responses.

Ipilimumab is approved for multiple indications including malignant pleural mesothelioma when in combination with nivolumab. The approved dose for this indication is 1 mg/kg every 6 weeks with nivolumab 360 mg every 3 weeks.

1.2.7 Rationale for Combination Therapy

Delayed and prolonged regressions in several mesothelioma patients treated with SS1P indicated anti-tumor immunity being induced by the immunotoxin[32]. The Pastan lab has constructed a murine breast cancer cell line expressing human mesothelin (hMSLN) (66C14-M) to investigate anti- tumor immunity induced by SS1P and LMB-100. These cells form tumors when implanted in one or more sites in BALB/c transgenic mice expressing hMSLN. The immunotoxins SS1P (first generation anti-mesothelin immunotoxin) or LMB-100 were injected directly into established tumors and anti-CTLA-4 was administered intra-peritoneally.

Mice implanted with two tumors, were injected with either SS1P or PBS in one of the two tumors. The other one was left uninjected. Mice treated with SS1P and anti-CTLA4 showed regression in 83.3% of the injected tumors (10 out of 12), in contrast to those treated with SS1P alone or anti-CTLA-4 and PBS. Furthermore 53% of the un-injected tumors had complete regressions demonstrating adding to the effect of antitumor immunity controlling tumor metastasis (Figure 5)[29].

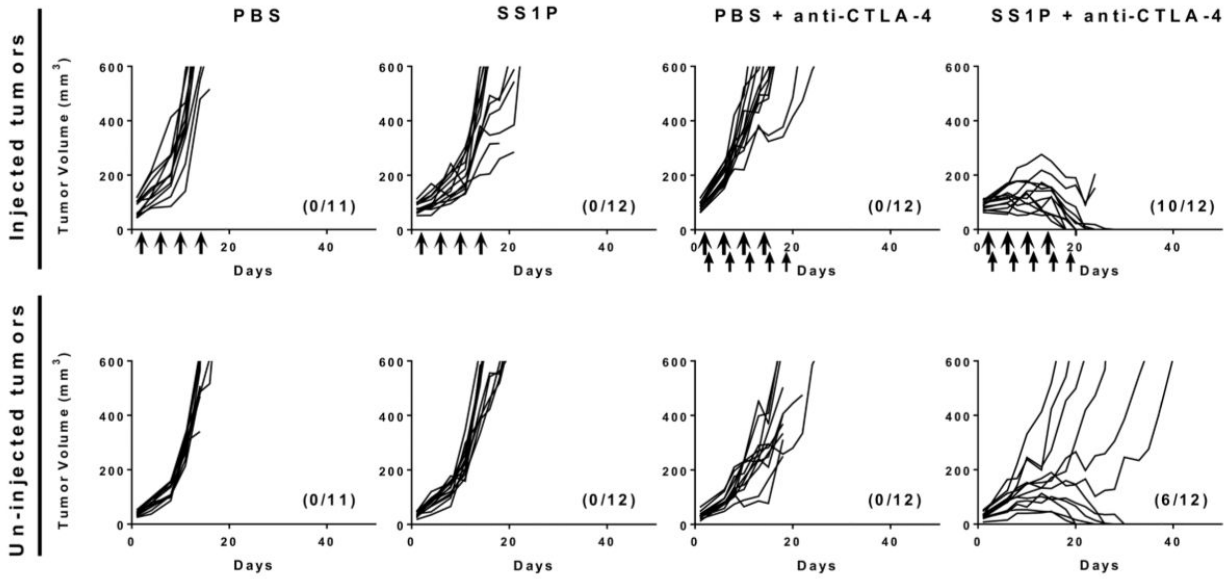


Figure 5. **Anti-CTLA-4 and SS1P eradicate injected tumors and uninjected tumors in a bilateral tumor model.** Individual growth curves of 66C14-M tumors implanted in two locations on the mice and treated with PBS (thick arrows) or 25 mcg SS1P (thick arrows) alone or in combination with anti-CTLA-4 (thin arrows). The injected tumors are represented in the upper panel and the uninjected tumors are in the lower panel. The number of mice in complete remission and total mice per group are shown in parentheses.

Combining anti-CTLA-4 with SS1P or LMB-100 induced complete tumor regressions in 16 out of 24 mice treated with the combination (67%). For LMB-100 specifically of the 13 mice treated, 11 (84%) had tumor regressions with 8 (61%) having complete tumor regressions (Figure 6**).**

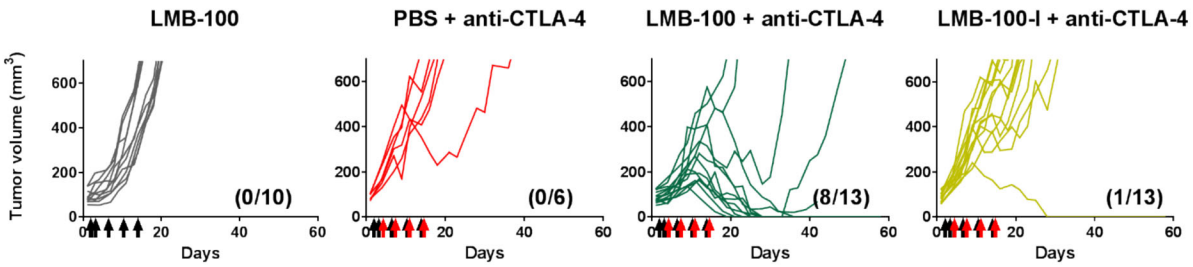


Figure 6: LMB-100 and anti-CTLA-4 produces complete remissions but inactive LMB-100 (LMB-100-I) does not. Tumor growth curves of individual mice treated with LMB-100 alone, PBS and anti-CTLA-4, 30 mcg LMB-100 and anti-CTLA-4, 30 mcg LMB-100-I and anti-CTLA-4. The number of mice in complete remission and total mice per group is shown in parentheses[29].

No tumor regression occurred, when treated with CTLA-4, SS1P, or LMB-100 alone. A non-toxic form of LMB-100 did not exhibit similar anti-tumor effect under these conditions, negating involvement of bacterial protein in producing anti-tumor immunity and demonstrating the vital role of mesothelin. Tumor regressions were associated with increased numbers of tumor infiltrating CD8+ cells. Administration of antibodies to CD8, greatly diminished immunotoxin efficacy (16% complete remission rate for LMB-100 + anti-CTLA4 + anti-CD8 antibodies), indicating the role of CD8+ cells in this anti-tumor effect. Surviving mice were protected from tumor re-challenge by 66C14 cells not expressing mesothelin, indicating the development of anti-tumor immunity[29].

In summary, these laboratory studies show remarkable synergy between intratumorally administered LMB-100 and immune checkpoint inhibition using anti-CTLA4 antibodies. Given the rationale for potentially increased anti-tumor effects following intratumor therapy and the better efficacy of intratumor LMB-100 and anti-CTLA4 antibody (compared to intravenous LMB-100), we plan to test the combination of intratumorally administered LMB-100 with ipilimumab in a Phase I study to assess feasibility and safety of this method

1.2.7.1 Rationale for sequential vs concurrent therapy (Amendment 10/17/2022)

On the current study, participants were to receive intratumorally administered LMB-100, beginning at dose level 1 (800mcg/cycle), in 21-day cycles. LMB-100 is given on days 1 and 4 and ipilimumab (3 mg/kg) given on day 2. Participants were to receive 2 cycles of LMB-100 plus ipilimumab, followed by 2 cycles of ipilimumab alone.

Two participants have been treated so far. The first patient, a 56-year-old man with pleural mesothelioma, received two cycles of treatment and experienced grade 2 hypoalbuminemia, however, did not experience a DLT. The second participant, a 68-year-old man with pleural mesothelioma, experienced rapid disease progression but was treated with one cycle of LMB-100 followed by ipilimumab. This patient experienced vascular leak syndrome, dyspnea, pericarditis, and hypoxia with pleural effusion which were all present at baseline but became worse after treatment around day 8. The PI decided the worsening pericarditis could possibly be attributed to the LMB-100. The participant developed grade 4 pericardial tamponade and grade 2 pneumonitis. After discussion with the Sponsor (OSRO), the event was considered possibly related to LMB-100 and classified as a DLT.

We suspect that high dose of ipilimumab administered along with LMB-100 could have resulted in toxicity observed in patients treated in this protocol so far. Hence, in order to reduce the occurrence of toxicity for patient safety we would like to administer one drug at a time. We think that this regimen would be better tolerated by the patients . We also believe it will still be efficacious as LMB-100 increases T-cell infiltration in the tumor and that these T-cells will still be present when ipilimumab is administered to respond to CTLA-4 blockade. and would still be efficacious as tumor cell infiltration.

For the above reasons, the protocol is being modified to one cycle of intra-tumor LMB-100 alone, followed by 3 cycles of ipilimumab at reduced dose (1mg/kg).

1.2.8 Rationale for Local Therapy

As systemic therapies have overall low response rates in MPM, it has long been suggested that combining aggressive local treatment with systemic chemotherapy might improve the outcomes. This idea is further supported by the localized nature of mesothelioma, being spread to adjacent organs and lymph nodes most frequently, with hematogenous spread being uncommon. Given the relatively ease of accessibility of the both the peritoneal and pleural disease, local treatments have been tried safely in the past with demonstrated safety profile. Furthermore, patients with mesothelioma have previously demonstrated strong responses to using localized treatments in both peritoneal and pleural mesothelioma.

Locally delivered immunotherapies have been demonstrated to cause site specific immune reactions within the tumor environment without the systemic exposure of the immunotherapy agents. This has been employed successfully in both bladder cancer and melanoma, with localized immunotherapy agents being approved for treatment of both diseases[33, 34]. This allows for a safer combination of different immunotherapies as well as the attainment of higher concentrations of the immunotherapies as well. There are several potential immunotherapy agents with locally administered PE toxins showing promise in Squamous Cell Head and Neck Cancer and Glioblastoma Multiforme [35, 36] [37]. Locally administered immunotherapies may be particularly effective in MM given its inherent tumor microenvironment, which contains milieu of immunosuppressive and fibroblastic cells, limiting delivery and efficacy of systemic immunotherapies [38-40]. Additionally, as the generation of tumor-specific immune responses is more critical than diffuse contact with the surfaces, local immunotherapies may lead to increased antigen exposure compared with systemic administration[12, 41].

Local studies conducted in the Pastan lab, supported the use of a local LMB-100 therapy from both a safety and efficacy standpoint. To evaluate our hypothesis that the anti-tumor efficacy of LMB-100 can be enhanced by anti-CTLA4 blocking antibodies, a 66C14 BALB/c mouse breast cancer cell line was transfected with a cDNA encoding human mesothelin to create the cell line 66C14-M. Because tumor cells expressing human mesothelin are rejected by normal BALB/c mice, the cells were grown in BALB/c mice expressing a human mesothelin transgene. The transgenic mice were found to express human mesothelin in the pancreas where mesothelin is not expressed in normal mice or humans. We found that some mice died when treated i.v. with 3 doses of 50 mcg of LMB-100, whereas non-transgenic (normal) mice were not killed by this dose. To avoid this toxicity, we chose to inject LMB-100 directly into 66C14-M tumors.

We found that the combination of LMB-100 injected directly into tumors with anti–CTLA-4 given i.p. causes complete regressions most of injected tumors and half of un-injected tumors and induces antitumor immunity.

Lastly given the restrictive and closed nature of the mesothelioma lesions, increased exposure in a closed space may also lead to strong localized immune responses.

1.2.9 Justification for Study Doses

1.2.9.1 LMB-100

The choice of dose levels is based on patient data from the completed Phase I study of LMB-100 rather than pre-clinical modeling. The MTD of single agent LMB-100 given intravenously (IV) has been established as 140 mcg/kg, on days 1, 3 and 5 of a 21-day cycle. In this study LMB-100 was diluted in line with 0.9NaCl so that the final concentration of the drug at time of administration into the bloodstream was 100 mcg/mL. We plan on conducting a 3 + 3 dose escalation trial (see Section 3.1.2) starting at an intra-tumor (IT) LMB concentration similar to that given IV in order to test the feasibility of this type of administration.

Although the total dose of LMB-100 to be given intratumorally is significantly less than the MTD, we believed that the local concentration of LMB-100 would be sufficiently high to kill tumor cells and initiate an immune response. In the first two participants, two cycles of LMB100 were planned for administration as it was anticipated that a majority of patients would develop neutralizing antibodies after 2 cycles of treatment with ipilimumab given for two additional cycles after the combination of LMB-100 plus ipilimumab for the first two cycles. Patient 1 received both cycles as planned, while patient 2 only received one cycle due to toxicity. However, it was noted that both the initial participants experienced toxicity (only one DLT) even at the very low dose administered. We have updated our strategy to reduce the number of cycles of LMB-100 administered from two to one. In addition, ipilimumab will not be administered in the same cycle as LMB-100.

1.2.9.2 Ipilimumab

Initially, ipilimumab was administered at a dose of 3 mg/kg every 3 weeks for up to 4 cycles. It was administered in combination with LMB-100 for the first two cycles. After the first two participants, the proposed dose is reduced to 1 mg/kg, the current FDA approved dose for ipilimumab in malignant pleural mesothelioma when given in combination with nivolumab. Ipilimumab will be administered in cycles 2-4 in order to clearly attribute any occurring toxicities to each study drug.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply.

2.1.1.1 Histologically confirmed malignant pleural or peritoneal mesothelioma not amenable to potentially curative surgical resection. The diagnosis will be confirmed by the Laboratory of Pathology, CCR, NCI.

2.1.1.2 Tumor must have epithelioid histology determined by the Laboratory of Pathology at the NCI. If the patient has biphasic histology, the epithelioid component must be >50%

2.1.1.3 Have provided archival tumor tissue sample or able to provide newly obtained core or excisional biopsy of a tumor lesion not previously irradiated. Formalin-fixed, paraffin

embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archived tissue.

Note: If submitting unstained cut slides, newly cut slides should be submitted to the testing laboratory within 14 days from the date slides are cut.

2.1.1.4 Have disease locally accessible disease to suitable for intratumoral injection of LMB-100.

2.1.1.5 Have measurable disease based on RECIST 1.1 (see Section 6.3). Lesions in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.

2.1.1.6 Subjects must have received prior immune checkpoint therapy with anti-PD-1/PD-L1 inhibitors alone or in combination with anti-CTLA4 blocking antibodies, as well as platinum based chemotherapy.

2.1.1.7 Age ≥ 18 years.

2.1.1.8 Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. See Appendix A. Evaluation of ECOG is to be performed within 28 days prior to initiation of study therapy.

2.1.1.9 Have adequate organ and marrow function as defined below:

System	**Laboratory Value**
Hematological	
– hemoglobin	≥ 9 g/dL[a]
– absolute neutrophil count	≥ 1,500/mcL
– platelets	≥ 100,000/mcL
Hepatic	
– total bilirubin	≤ 2.5 X institutional ULN OR direct bilirubin ≤ ULN for participants with total bilirubin levels >1.5 X ULN
– AST and ALT	≤ 2.5 X institutional ULN (≤ 5 X ULN for participants with liver metastases)
Renal	
– Creatinine OR – Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥ 50 mL/min for participant with creatinine levels > 1.5 X institutional ULN
Coagulation	

– International normalized ratio (INR) OR prothrombin time (PT) – Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal. a. Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks. b. Creatinine clearance (CrCl) should be calculated per institutional standard.	

2.1.1.10 Must have left ventricular ejection fraction >50%.

2.1.1.11 The effects of LMB-100 on the developing human fetus are unknown. For this reason and because ipilimumab is a Category C agent, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) while on study therapy and for four months after the last dose of study therapy. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

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

2.1.2 Exclusion Criteria

2.1.2.1 Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 3 weeks prior to initiation of study therapy.

Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 3 weeks after the last dose of the previous investigational agent. Patients with active devices will be excluded from the study

2.1.2.2 Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e. without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to initiation of study therapy.

2.1.2.3 Has active systemic issues as bleeding diathesis or active infections

2.1.2.4 Presence of a clinically significant pericardial effusion

2.1.2.5 Has severe hypersensitivity (≥Grade 3) anti-CTLA4 therapies and/or any of their excipients.

2.1.2.6 Has received prior radiotherapy to the site of local administration

2.1.2.7 Subjects who have received LMB-100 previously

2.1.2.8 Has received prior systemic anti-cancer therapy including investigational agents within 3 weeks prior to initiation of study therapy. Patients who have received prior anti-PD-1/PD-L1 or CTLA4 antibodies are eligible. Any toxicity related to these agents must have resolved to grade 1 and they must not be on systemic immunosuppressive therapies (physiologic dose of steroids are permitted).

2.1.2.9 Has received prior radiotherapy to site other than target lesion within 2 weeks prior to initiation of study therapy. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.

2.1.2.10 Has not recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible. If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to initiation of study therapy.

2.1.2.11 Has received a live vaccine within 30 days prior to initiation of study therapy. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

2.1.2.12 Is receiving therapeutic anti-coagulation. Patients receiving prophylactic anticoagulation may be eligible if in the opinion of the study team, anti-coagulation may be stopped during the time of LMB-100 administration and tumor biopsies

2.1.2.13 Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to initiation of study therapy.

2.1.2.14 Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g.., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

2.1.2.15 Has a QTcF interval >480 milliseconds

2.1.2.16 Has a history of (non-infectious) pneumonitis/interstitial lung disease(ILD) that required steroids or has current pneumonitis/ILD

2.1.2.17 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

2.1.2.18 Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

2.1.2.19 A woman of childbearing potential who has a positive pregnancy test within 72 hours prior to initiation of study therapy. If the using a urine test and test positive or cannot be

confirmed as negative, a serum pregnancy test will be required. **Note:** in the event that 72 hours have elapsed between the screening pregnancy test and the first dose of study treatment, another pregnancy test (urine or serum) must be performed and must be negative in order for subject to start receiving study medication.

2.1.2.20 Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 4 months after the last dose of trial treatment. Pregnant women are excluded from this study because LMB-100 + ipilimumab are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with LMB-100 + ipilimumab, breastfeeding should be discontinued if the mother is treated with LMB-100 + ipilimumab. These potential risks may also apply to other agents used in this study.

2.1.2.21 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. .

2.1.2.22 Positive for Hepatitis B or C (defined as Hepatitis B surface antigen reactive) or known active Hepatitis C virus (defined as HCV RNA detected) infection. or active HBV or HCV infection.

2.1.2.23 Has a known additional malignancy that is progressing or has required active treatment within the past 2 years. Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.

2.1.2.24 Has an active infection requiring systemic therapy.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Subjects will also be drawn from patients seen at the mesothelioma clinic at the NIH Clinical Center as well as from referrals from outside providers.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which

screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Performed at any time prior to initiation of study therapy

- 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.

Performed within 28 days prior to initiation of study therapy

- History and physical exam including height and weight
- Vital signs including pulse oximetry
- ECOG performance status
- Electrocardiogram (ECG)
- Echocardiogram
- CT scan of chest, abdomen and/or pelvis and areas of known or suspected disease involvement. MRI with or without gadolinium may also be used if CT scan not feasible.
- FDG-PET scan
- Urinalysis
- HIV antibody testing
- Hepatitis B and C screen

Performed within 10 days prior to initiation of study therapy

- 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), lactate dehydrogenase, Thyroid (TSH, free T4, total T3), uric acid

Performed within 72 hours prior initiation of study therapy

- Urine or serum hCG in women of childbearing potential

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a laboratory abnormality may be rescreened.

2.4 TREATMENT ASSIGNMENT PROCEDURES:

Cohorts

Number	Name	Description
1	mesothelioma	Subjects with pleural or peritoneal mesothelioma

Arms

Number	Name	Description
1	Intratumoral LMB-100 Administration	Those with pleural or peritoneal mesothelioma receiving intratumoral administration of LMB-100 + ipilimumab for up to 4 cycles

Stratifications, Randomization and Arm Assignment

No stratification of randomization will occur on the study.

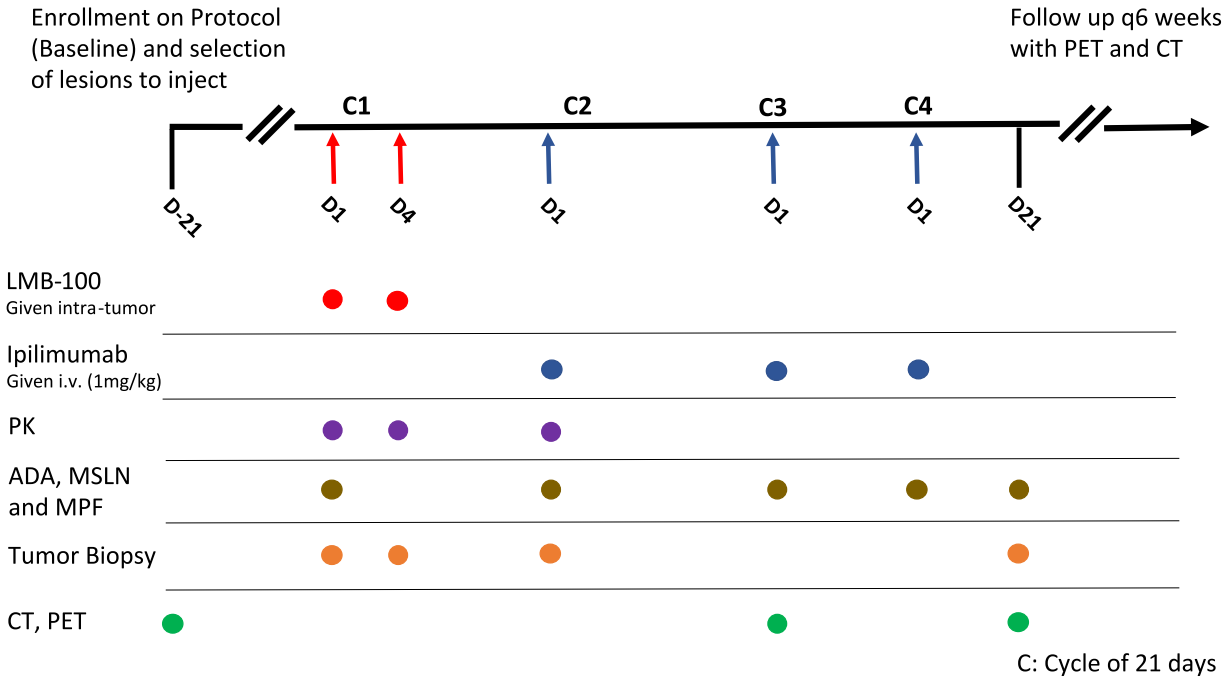
Subjects in cohort 1 will be directly assigned to arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single center Phase I study of intratumorally administered LMB-100 in combination with ipilimumab in patients with advanced pleural and peritoneal mesothelioma who have progressed on standard therapies. We will test feasibility and safety of LMB-100 injected through the intratumoral route. We will use a standard, 3+3 dose-finding study to demonstrate safety and observe anti-neoplastic activity of intratumorally administered LMB-100 therapy with a standard dose regimen of IV ipilimumab. Patients will receive LMB-100 beginning at dose level 1, on days 1 and 4 of the first 21-day cycle. Ipilimumab will be administered on day 1 of cycles 2, 3 and 4 at a dose of 1 mg/kg. Hospitalization is required during cycles when LMB-100 is administered and is expected to last for about 8 days, though duration may vary as clinically indicated. Hospitalization is not required during cycles when LMB-100 will not be administered. Tumor biopsies will be performed prior to each LMB-100 administration, prior to ipilimumab administration on C2D1 and at completion of study therapy to evaluate changes in the tumor immune microenvironment.

3.1.1 Schema



3.1.2 Dose Escalation

The first 3 patients will start at dose level 1 which is equivalent to the drug concentration administered during clinical investigations for IV LMB-100. There will be a total of 2 dose levels. In the event that dose level 1 is not tolerated by the first dosing group (i.e., more than 2 of 3 have DLT), dose level will be reduced to dose level -1. Table 4 highlights the drug levels. In the event that dose level 1 is not tolerated by the first dosing group (i.e., more than 2 of 3 have DLT), dose level will be reduced to dose level -1.

Each dose-escalation or de-escalation decision will be documented in the study file. The report including the supporting safety data and delineation of each criteria met, with the dose-escalation/de-escalation decisions will be provided to and approved by the Sponsor (OSROSafety@nih.gov) before additional participants will initiate study therapy. The Dose Escalation Determination form on the sponsor website:

https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions may be used for this purpose.

Table 4: Dose levels

Dose Level	Concentration of IT LMB-100 (mcg/mL)	Volume (mL)¶	LMB-100 administered per dose (mcg)+	LMB-100 administered per cycle (mcg)	IT dose as % of the IV MTD (mcg/kg/cycle)*/§
-1	50	4	200	400	1.36%
1	100	4	400	800	2.72%
2	300	4	1200	2400	8.16%

IT, intra-tumoral; IV, intravenous

¶maximum injected volume of diluted LMB-100

+dose administered on days 1 and 4 of 21-day cycle, for 1 cycle

* IV MTD is 420 mcg/kg/cycle, split in 3 doses of 140 mcg/kg/dose given IV on days 1, 3 and 5

§based on average body weight of 70 kg, i.e., 29.4 mg/cycle of IV dose

All subjects will be observed for dose-limiting toxicity (DLT) until the end of cycle 2. An adverse event that occurs is a DLT, if it meets the definition in Section 3.2.

Dose escalation decisions will be based on the following:

- If no subject (0/3) experiences a DLT, then enrollment into that dose level is closed and subsequent subjects may be enrolled into the next higher dose level.
- If one (1/3) subject experiences the DLT then the dose level will be expanded to 6 subjects. If none of these additional subjects experience DLT (i.e., 1/6 with DLT), the dose will be escalated.
- If ≥2 subjects at any (3-6 subject) dose level experience the DLT then the MTD is exceeded, and enrollment in that dose level must cease. The next lower dose level will be expanded to 6 subjects (if it does not already contain 6 subjects). If ≤1 subject experiences the DLT then that dose level is the MTD.

3.1.3 Definition of the MTD

The MTD will be defined as follows:

- The dose at which ≤ 1 of 6 subjects experienced DLT
- The dose level immediately below the level at which > 1 out of 3-6 subjects experienced DLT

The MTD will be the recommended phase 2 dose (RP2D)

3.2 DOSE LIMITING TOXICITIES

Only those adverse events deemed suspected of a relationship to LMB-100 will be used in the definition of DLT. Adverse Events that are considered disease-related (not suspected of relationship to LMB-100) will not be considered dose-limiting toxicities. The definition of DLT in these studies uses NCI's Common Terminology Criteria for Adverse Events (CTCAEv5.0). The following adverse events that are suspected of causal relationship to LMB-100 or ipilimumab during cycles 1 and 2 will be considered DLTs:

- Any Grade 4 hematological toxicity (with the exception of lymphopenia) lasting greater than five days will be considered a DLT. Lymphocyte count and subsets will not be considered in the definition of DLT.
- Grade 3 febrile neutropenia (ANC <1.0 x 10^9 cells / L with a single temperature of >38.3°C or a sustained temperature of ≥38°C for more than one hour
- Grade 3 thrombocytopenia associated with bleeding episodes
- Any Grade 3 or greater, non-hematological toxicity with the following **exceptions**:

- Tumor lysis syndrome, including associated abnormalities (e.g., electrolytes, uric acid, renal function)
- Grade 3 electrolyte changes unless associated with clinically significant consequences. Any Grade 4 levels will be considered a DLT. Hypocalcemia toxicity grade should be assigned based on the calcium level corrected for degree of hypoalbuminemia
- Grade 3 nausea and diarrhea which has not received appropriate treatment;
- 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
- Alopecia
- 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 3.3.1.4).
- Asymptomatic ≥ Grade 3 lymphopenia, leukopenia, hypoalbuminemia, electrolyte abnormalities resolving within 24 hours, increase in alkaline phosphatase.

If a patient did not experience DLT and did not finish the first two cycles, he or she will not be evaluable for DLT 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 -1, an amendment may be submitted in order to explore lower dose levels.

3.3 DRUG ADMINISTRATION

3.3.1 LMB-100

The qualified health care professional responsible for dispensing the study drug will prepare the correct dose according to the dose level allocation of each patient. LMB-100 will be given intratumorally as detailed in Section 3.3.1.2. LMB-100 must be administered in a hospital in an interventional radiology suite by a trained interventional radiologist. 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.

3.3.1.1 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.

3.3.1.2 Intratumor Injection of LMB-100

LMB-100 will be administered by a trained interventional radiologist following an ultrasound-or CT-guided intralesional injection method similar used for intratumoral injection of the FDA approved IMLYGIC™ (Talimogene Laherparepvec)[14]. Lesions may be pleural, subcutaneous, visceral and any other location that is deemed safety accessible for injection by a trained interventional radiologist. Upon enrollment, lesions to be injected will be selected by the primary team in conjunction with the interventional radiology department at the NIH Clinical Center.

On day of administration, patient will have lesions to be injected validated by the primary team. After confirmation, patient will have anesthesiology evaluation for method of sedation for the procedure. The patient will then undergo sedation (general anesthesia, conscious sedation) at the discretion of anesthesiology followed by topical anesthesia to the areas to be injected. Once sufficient anesthesia is provided, injections will proceed.

Injections will be carried out typically using a sterile 18 to 22 gauge needle (. The size of the needle will depend on the lesion's characteristics. The needle will be placed in the center of the lesion and 4 mL of the appropriate concentration of LMB-100 (see Table 4) will be delivered using sterile techniques. Drug delivery may occur across multiple lesions and will be performed under ultrasound or CT-guidance. For each lesion injected, the needle may be redirected along multiple tracks as far as the radial reach of the needle allows to achieve complete dispersion within the lesion. Multiple insertion points may be used if a lesion is larger than the radial reach of the needle. Lesions may have elevated oncotic pressure that resists intratumoral injection, and therefore the volume must be delivered slowly and gradually at IR discretion. The exact duration of the injection will be recorded. The needle should always be withdrawn from the skin slowly (allowing a period of 15–30 seconds from the end of injection before initiating needle withdrawal) to minimize the risk of leakage of injectate back through the puncture site.

3.3.1.3 Premedications for Patients Receiving LMB-100

Due to the prevalence of infusion related reactions (IRRs) seen in the previous study of IV 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 (or alternative antihistamine at adequate dose)
- Famotidine 20 mg PO or IV (or alternative H2 blocker at adequate dose)
- Acetaminophen 650 mg PO or IV

(See section 3.4.1 for complete instructions on response to IRRs)

3.3.1.4 Additional Precautions at Infusions of LMB-100 after Cycle 1 Day 1

Participants having experienced an IRR of Grade 2 to 4 on a previous infusion despite standard pre-medication should also receive:

- 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 3.4 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.

3.3.1.5 Post injection monitoring

The patient will return to the inpatient unit for monitoring. Patient will be restricted to their bed and monitored with post injection vital signs per Study Calendar.

We anticipate inflammatory states within the tumor. Based on our experience with intravenous administration we have seen tumor pain manifest. As a result, we will diligently monitor the state of the patient's pain and accordingly treat.

3.3.2 Ipilimumab

Participants not experiencing DLT during cycle 1 will begin ipilimumab in cycle 2 as long as one dose of LMB-100 has been completed. Ipilimumab 1mg/kg will be administered as a 90-minute IV infusion on Day 1 of cycles 2, 3 and 4. Every effort will be made to target infusion timing to be as close to 90 minutes as possible. However, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 90 minutes: -5 min/+10 min). Ipilimumab may be administered on an outpatient basis. The package insert contains specific instructions for the preparation of the ipilimumab infusion fluid and administration of infusion solution. Post infusion monitoring of vital signs will occur per Study Calendar.

3.4 Dose Modification

3.4.1 LMB-100

LMB-100 injection may be held for up to 96 hours due to drug-associated toxicity or adverse events from other intercurrent medical conditions (such as primary cancer diagnosis) that resolve with or without medical intervention to grade 2 or less within this time frame. Toxicities for which further LMB-100 treatment should NOT be given or which are exceptions to the above guidelines are stated below in Table 5. The table also provides guideline on how to manage some toxicities anticipated with LMB-100.

Table 5. 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

Event	Action to Be Taken
	saline, oxygen, bronchodilators, corticosteroids, and vasopressors depending on the symptoms. If the infusion is interrupted: o 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). o 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. o 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 o Patients experiencing IRR CTCAE Grade 4 or anaphylaxis must be permanently discontinued from LMB-100 treatment

Event	Action to Be Taken
Capillary leak syndrome	In the event of Grade ≥2 CTCAE capillary leak syndrome (medical intervention indicated): o Delay LMB-100 administration until complete resolution of the event o For hypotension minimize fluid resuscitation to avoid fluid overload Minimize crystalloid solutions (e.g., saline) o Vasopressor support (e.g., phenylephrine) if indicated to stabilize blood pressure o 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 o For pulmonary congestion provide diuretic and/or albumin treatment in case of hypoalbuminemia as appropriate o Progressive shortness of breath may require in addition endotracheal intubation or drainage of a pleural effusion o For oliguria and /or rising serum creatinine level delay LMB-100 if Grade C3 urine output (<10 mL/hr) o Use fluids judiciously if increase in urine output is required o Use dopamine if patient is unresponsive to or unable to tolerate fluids Monitor serum albumin levels prior to the LMB-100 treatment cycle o 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	o Hydrocortisone (200 mg IV) or equivalent dose of another corticosteroid as clinically indicated o 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

Event	Action to Be Taken
	○ In the event of pleuritis resulting in mild to severe pleuritic pain, treat with analgesics or steroids as clinically indicated ○ 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	In the event of Grade 1 or greater renal toxicity consider increasing oral or intravenous hydration, and consider delaying LMB-100 administration by up to 72 hours. In the event of Grade 2 or greater renal toxicity hold LMB-100 administration until recovery to Grade 1 or better. If this does not occur within 72 hours, no further LMB-100 should be given during the cycle
IRR = infusion related reaction; IV = intravenous; CTCAE = Common Terminology Criteria for Adverse Events	

3.4.2 Ipilimumab

3.4.2.1 Dose delay/discontinuation and toxicity management for AEs associated with ipilimumab

Please refer to the latest version of the ipilimumab label found on the FDA website (https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=BasicSearch.process) for toxicity management.

In general, no dose reduction for ipilimumab is recommended. In general, withhold ipilimumab for severe (Grade 3) immune-mediated adverse reactions. Permanently discontinue ipilimumab for life-threatening (Grade 4) immune-mediated adverse reactions, recurrent severe (Grade 3) immune-mediated reactions that require systemic immunosuppressive treatment, persistent moderate (Grade 2) or severe (Grade 3) reactions lasting 12 weeks or longer after last ipilimumab dose (excluding endocrinopathy), or an inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks of initiating steroids.

3.4.2.2 Other allowed dose interruption for ipilimumab

Ipilimumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

3.5 On Assessments Performed Outside of the NIH Clinical Center

The protocol allows for the following assessments to occur outside of the NIH Clinical Center as indicated in the individual sections and in the study calendar.

1. Screening assessments (section 2.2.2) performed within the appropriate timeframes are permitted outside of the NIH in order to minimize participant discomfort. All tests required at screening are commonly performed and utilize similar methodologies across testing centers.

2. Day 15 assessments (section 3.6) include standard laboratories which in general use the same methodology across all laboratories. These labs and are performed as part of a general safety check and are not relied upon solely in deciding whether to continue or discontinue study therapy.

3. Follow up assessments ((section 3.6) include standard laboratories and CT scans/MRIs. These assessments will be used in the determination of safety endpoints as well as efficacy endpoints. However, given that the methodologies utilized are similar across all laboratories, no impact on the study data are anticipated from this use.

Remote visits will be conducted in compliance with NIH guidelines and FDA regulations.

3.6 STUDY CALENDAR

Screening assessments will occur within 28 days prior to start of study therapy unless otherwise indicated. If screening assessments are performed within 3 days prior to dosing, the assessments do not need to be repeated on C1D1 unless otherwise indicated.

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

1 cycle = 21 days

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

Procedure	Screening	Cycle 1								Cycles 2, 3 & 4	Cycle 4	Follow-Up Visit (4-6 weeks after EoT)[14]	Long-Term Follow Up (every 6 – 12 weeks)[16]
		Day								Day 1	Day 21 (±7 days)		
		1	2	3	4	5	7	8	15[13]				
LMB-100		X			X[1][1]								
Ipilimumab										X			
History and PE	X	daily during hospitalization								X		X	
Weight	X	daily during hospitalization								X		X	
Height	X												
Vital signs[1]	X	at least daily during hospitalization. Additional timepoints around drug administration[1]								X		X	
Performance Score	X	X								X			

Procedure	Screening	Cycle 1								Cycles 2, 3 & 4	Cycle 4	Follow-Up Visit (4-6 weeks after EoT)[14]	Long-Term Follow Up (every 6 – 12 weeks)[16]
		Day								Day 1	Day 21 (±7 days)		
		1	2	3	4	5	7	8	15[13]				
Viral screen (HIV, Hepatitis B, Hepatitis C)	X												
Labs[2]	X[7]	X[9]		X		X		X	X	X		X	
ACTH, TSH, free T4, total T3, CA125		X								X			
Urinalysis	X	X								X			
HLA Typing (Class I and Class II)		X[10] C1 only											
Urine or serum hCG in women of childbearing potential[3]	X[8]	X[8]								X		X	
Confirmation of dx incl biopsy if archival material insufficient[4]	X												
Biopsy (as feasible)		X			X[11]					X (cycle 2)	X[12]		

Procedure	Screening	Cycle 1								Cycles 2, 3 & 4	Cycle 4	Follow-Up Visit (4-6 weeks after EoT)[14]	Long-Term Follow Up (every 6 – 12 weeks)[16]
		Day								Day 1	Day 21 (±7 days)		
		1	2	3	4	5	7	8	15[13]				
Correlative Research Studies		Please see section 5.2											
CT CAP or MRI [5]	X	at the end of cycles 2 & 4 ± 7 days										X[15]	X
FDG-PET	X	at the end of cycles 2 & 4 ± 7 days										X[15]	X
ECG[6]	X	X								X		X	
Echocardiogram	X												
Adverse Events		Monitored continuously											
Concomitant Medications		Monitored continuously											
Remote check in q 12 weeks													X

[1] **At screening:** heart rate, blood pressure, body temperature, pulse oximetry. **Pre and Post LMB-100 injection**, vital signs (heart rate, blood pressure, body temperature, pulse oximetry) have to be monitored every 30 minutes (±10 minutes) after injection for the first hour, hourly (±10 minutes) for the next 4 hours after injection, then approximately every 8 hours while hospitalized. **Post Ipilimumab infusion**, vital signs will be monitored approximately every 8 hours while hospitalized. During cycles 3 & 4 if patient not hospitalized, vital signs performed every 30 minutes (±10 minutes) after infusion for one hour.

[2] CBC with differential, Acute Care Panel (Na, K, Cl, CO2, creatinine, glucose, BUN), Hepatic Panel (ALP, AST, ALT, total & direct bilirubin), Mineral Panel (albumin, Ca, Mg, P), lactate dehydrogenase, creatine kinase, C-reactive protein, PT, PTT, fibrin degradation products, total protein, uric acid.

[3] Required in women of childbearing potential.

[4] Please see section 2.2.2 for tissue requirements

[5] CT scans are preferred modality. However in lieu of CT scans, MRIs with or without gadolinium may be performed if patient is unable to undergo CT scanning.

[6] Single 12-lead ECG will be recorded at screening, then pre- and end (+30 minutes) of LMB-100 infusion for first cycle 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.

[7] Performed within 10 days prior to initiation of study therapy.

[8] Performed within 3 days prior to initiation of study therapy. Must be repeated on C1D1 (pre-treatment) if more than 72 hours have passed since screening assessment and actual treatment initiation.

[9] For cycle 1, only required if more than 10 days have passed since screening assessment. If eligibility criteria are not met at this timepoint, subject may not be treated.

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

[11] Day 4 LMB-100 administration and associated biopsy may be performed within one day of indicated time based on interventional radiology availability.

[12] If study therapy discontinued before C4D21, the biopsy may be performed at discontinuation as long as at least one dose of ipilimumab was administered.

[13] Day 15 laboratory assessments may be performed by certified labs outside of NIH if participant unable to travel.

[14] EoT = end of therapy. 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 with a focused medical history will be performed by telephone or other NIH approved remote communication platform used in compliance with local policy, including HRPP policy 303 and the listed clinical and laboratory assessments may be requested from an outside provider with the following exceptions: physical examination, including height, weight and vital signs. The excepted assessments may be omitted. If they are performed at an outside site as part of standard of care, we will request the records. Should the focused medical history uncover an issue, participant will be referred for follow up with an outside physician. These records will also be requested.

[15] Performed at 4 weeks after the end of final treatment cycle if patient did not have progressive disease

[16] Scans performed only in patients who have not had progressive disease. Scans will continue every 6 weeks until disease progression or start of a new anti-cancer treatment (CT scans/MRIs from outside facilities are acceptable. PET scans will not be required if participant unable to return to NIH). After disease progression, subjects will be followed every 12 weeks by telephone/email/other NIH approved remote communication

platform used in compliance with local policy, including HRPP policy 303 for assessment of survival status, adverse events and initiation of new anti-cancer therapy

3.7 COST AND COMPENSATION

3.7.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.7.2 Compensation

No compensation is provided for participants on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.8.1 Criteria for removal from protocol therapy

- Completion of protocol therapy
- Disease progression (confirmation scan required)
- Noncompliance with study treatment or procedure requirements
- Requirement for use of prohibited medications/vaccines (see 4.1.2)
- Participant (or LAR if one was designated when participant become incapacitated during study) requests to be withdrawn from active therapy
- Permanent loss of capacity to consent if subject not benefiting from study therapy as described in section 11.3
- Unacceptable toxicity as defined in sections 3.2 and 3.4
- Investigator discretion
- Positive pregnancy test

3.8.2 Off-Study Criteria

- Investigator decision to end the study
- Participant requests to be withdrawn from study
- Lost to follow up
- Screen failure

- Death

3.8.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 24 hours and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs documented beyond this point.

4.1.1 Permitted Therapy

All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

Unless otherwise specified in section 3.4, participants will be managed per current evidence-based practice guidelines in consultation with a medically responsible investigator if available.

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 while patient is receiving study therapy:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than the study agents

- Radiotherapy. Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be considered per investigator discretion.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study therapy is required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

Please note that tubes and media referenced in sections 5.1 and 5.2 below may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1 BIOSPECIMEN COLLECTION

5.1.1 Pharmacokinetic Assessments

All blood samples for PK assessment will be collected from an IV line. 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 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. Ludmila Krymskaya 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)

The generation of a humoral response to LMB-100 may limit subsequent cycles by the development of anti-drug antibodies. In this study we aim to correlate anti-drug antibody formation post LMB-100 injection with disease response on imaging as well as on maximal drug concentrations. Anti-drug antibodies will be retrospectively evaluated using a validated ELISA assay. We will also be assessing anti-drug antibody formation against ipilimumab using a validated electro-chemiluminescence assay on the Meso Scale Discovery platform.

5.1.2.1 Sample Collection

Samples will be per the schedule in 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 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 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. Ludmila Krymskaya 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 Retrospective Analysis of Tumor Mesothelin Expression

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 timepoints in section 5.2 may be used for this purpose. Specimens will be used to correlate treatment with response with mesothelin expression in exploratory analyses.

5.1.3.1 Specimen collection

Collection of tumor biopsies where feasible and deemed safety by the multidisciplinary review team 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. Typically at least 2, ideally 4 core biopsies will be obtained at each time point. 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. Pain management for biopsy collection will include local anesthesia and conscious sedation. However, as the biopsies in collected in cycle 1 will be done at the same time as tumor injection, separate anesthesia for

biopsies is unlikely at that time as the anesthetic method used for the tumor injection will still be effective.

5.1.4 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.

5.1.4.1 Sample Collection

Samples will be obtained prior to the first LMB-100 dose in cycle 1 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

5.1.4.2 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.

5.1.4.3 Sample Storage

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

5.1.5 Gene Expression-Based Characterization of the Immune Landscape Before and After Treatment with LMB-100 and Ipilimumab

Tumor biopsies will be collected per section 5.2. Tissue collected at screening (including archival) may also be used. We will evaluate tumor biopsies before and after treatment with LMB-100 and before and after treatment with ipilimumab 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 and ipilimumab. 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.

5.1.6 Tumor Microenvironment Studies Using Multiplex Staining Technologies

Tumor biopsies will be collected per section 5.2. Portions of the tumor tissue collected at each timepoint and stored in the Hassan lab will be provided to the CAT-I, the laboratory of Dr. Ronald Germain. The tissue will be analyzed for cancer associated immune biomarker alterations including the immune cell and tumor cell co-localization with confocal microscopy and histo-cytometry. The CAT-I lab will return confocal images and quantitative analyses of these images in figure format to the Hassan lab.

Samples will be coded in the laboratory of Dr. Raffit Hassan and sent to CAT-I for imaging studies using multiplex staining technologies. The code key will be retained by individuals in the Hassan laboratory and will not be provided to members of the CAT-I lab. Results will be returned to study investigators.

5.1.7 Assessment of plasma cytokine levels

LMB-100 may induce systemic inflammatory response and cause cytokine release syndrome. We will be assessing IFN-γ, IL-1β, IL-6 and TNF-α levels in plasma using a validated electro-chemiluminescence assay on the Meso Scale Discovery platform. This assay quantifies cytokine

levels in human plasma using the V-Plex electrochemiluminescence multiplex platform manufactured by Meso Scale Discovery (MSD).

5.1.7.1 Sample Collection

Samples will be per the schedule in section 5.2

Draw 2mL into K2EDTA 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 collection is highly preferred.

5.1.7.2 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 specimen to 2mL cryovials and store at -70°C.

Cytokine levels will be assessed on retrospectively.

5.1.7.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to Dr. Ludmila Krymskaya 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

5.1.7.4 Sample Storage

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

5.2 SAMPLE COLLECTION SCHEDULE

Cycle	Day	**PK**[a] (5.1.1)	**ADA** (5.1.2)	**Tumor Sample** (5.1.3, 5.1.5, 5.1.6)	**Serum mesothelin and MPF** (5.1.4)	**Plasma cytokine** (5.1.7)
		2 mL K_2EDTA tube	2 mL K_2EDTA tube	NA	2 mL blood in 3.5 mL tiger top tube	2 mL K_2EDTA tube
Screening	Screening period ± 3 days			X[b]		
1	1	Pre-dose, 1, and 4 hours after end LMB-100 administration	Pre-dose	Pre-dose[c]	X	Pre-dose
	4	Pre-dose, 1, and 4 hours after end LMB-100 administration		Pre-dose[c]		Pre-dose
	7 (± 2 days)					X
2	1	Pre-dose (ipilimumab)	Pre-dose	Pre-dose[c]		
3 & 4	1		Pre-dose			
End of Treatment C4 (D21)			X	X[d]	X[d]	

a. Timed samples, including EOI, will be collected at indicated timepoints -15/+30 minutes

b. Archival or if not available, a fresh Biopsy or Tumor Effusion.

c. Biopsies– collected as feasible with each dose of LMB-100, prior to the first dose of ipilimumab and after the last dose of ipilimumab. Note day 4 drug administration and therefore biopsy collections may occur within ± one day of indicated time.

d. EOT biopsy may be collected within (±) 7 days of C4D21. If EOT occurs earlier than the end of cycle 4, an option is provided to collect a biopsy at treatment discontinuation as long as at least one dose of ipilimumab has been administered.

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 Blood Processing Core

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab 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 the 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 or -80°C according to stability requirements. These freezers are located onsite in the BPC 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 BPC. 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 de-identified of personal data, with access 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-Core Laboratories in 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 termination 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, the proposal will be submitted to IRB or OHSRP for review. 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 NCI Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissue that is not placed in paraffin blocks is stored in formalin for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB/OHSRP approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.3.4 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 are tracked by distinct identification labels generated by Labmatrix that include a unique patient identifier and date of specimen collection. Thus samples will be de-identified of personal data, with access 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)

5.3.5 CAT-I Laboratory

The CAT-I laboratory (CAT-I) will obtain coded samples from the Hassan laboratory. Upon acquisition, members of the CAT-I laboratory will enter these samples into CEREBRO, an advanced sample labeling system that tracks each sample through every step of the workflow. To meet requirements for availability of primary data and for quality assurance checks, CAT-I will maintain a detailed inventory of the type (slide, paraffin block, tissue, frozen OCT block) and location of each sample in the laboratory. More specifically, paraffin blocks and accompanying slides will be maintained at room temperature in the CAT- I laboratory. Tissues provided by the Hassan laboratory will be fixed, frozen, and stored in the CAT-I's -80°C freezer. The CAT-I laboratory will be locked when CAT-I lab members are not present. Unprocessed samples will be

held by the CAT-I or returned to the Hassan laboratory upon their request. Finally, CAT-I will comply with requirements for annual Biospecimen Reporting at the NIH.

5.3.6 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.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR 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.

Record AEs from the first study intervention, study day 1, through 30 days after removal from study treatment. Adverse events occurring more than 30 days after the last dose of study therapy are required to be recorded only if they are considered to be serious and related to the investigational agent.

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, will be reported expeditiously per 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:

- De-identified data in an NIH-funded or approved public repository.
- De-identified data in BTRIS (automatic for activities in the Clinical Center)
- De-identified or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

- Data will be shared through*:*
- An NIH-funded or approved public repository. Insert name:_clinicaltrials.gov.
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- 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 per Study Calendar. 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)[42] 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)[43] should be used as described in section 6.3.2.

iRECIST, based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs, will also be utilized for pleural mesothelioma and peritoneal mesothelioma as follows.

When the Investigator identifies radiographic progression per RECIST 1.1 or mRECIST, efforts should be made to verify radiologic PD. Treatment should continue until PD has been verified. Regardless of whether PD is verified, if the Investigator considers the participant has progressed, but elects to implement iRECIST, the Investigator will assess for confirmation of progression by iRECIST at subsequent time points. When clinically stable, participants should not be discontinued until progression is confirmed by the Investigator, working with local radiology. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some participants can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response.

6.3.1 Peritoneal Mesothelioma

6.3.1.1 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: ≥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.

6.3.1.2 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[44-46]. 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[47].

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.

6.3.1.3 RECIST version 1.1 Response Criteria

6.3.1.3.1 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.

6.3.1.3.2 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).

6.3.1.3.3 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)

Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR

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

6.3.2 Pleural Mesothelioma

Malignant pleural mesothelioma (MPM) lesions are difficult to measure reliably[43]. Therefore, modified criteria were defined in 2004 adjusting target lesion measurements to the specific needs of this disease.

6.3.2.1 Modified RECIST Criteria for Pleural Mesothelioma

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.

6.3.2.2 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.

6.3.3 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.

6.3.4 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.

6.3.5 Objective Response Rate

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

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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

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 at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported t 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 at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

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

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

7.4 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 in the appropriate timeframes.

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.

8 SPONSOR PROTOCOL/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 5.

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 with the exception of any listed in section 8.4.

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. Any exceptions to the expedited reporting requirements are found in section 8.4.

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. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

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 Waiver of expedited reporting to CCR

As death/hospitalization due to disease progression are part of the study objectives (OS, ORR) and captured as an endpoint in this study, they will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section 8.3.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

NA.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov . Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal exposure

If a patient becomes pregnant while still under study requirement to use contraception, the study treatment should be discontinued immediately if participant still on study therapy, and the pregnancy must be 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 become 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 occurring from the date of the first dose of study therapy until 4 months after the last dose should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of study therapy.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 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.

8.8 SPONSOR DATA AND SAFETY MONITORING BOARD

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including a biostatistician experienced in statistical methods for clinical trials and a clinician with relevant expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At that time, each data element that the DSMB needs to assess will be clearly defined. Following review of the study, the DSMB will provide its recommendations, signed by the Committee Chair and OSRO Director, which are then distributed to the PI for the study under review.

8.9 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements. In addition, any deviation to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and
- that the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

10.1.1 Primary Objectives

- To determine safety and feasibility of intra-tumoral LMB-100 injection plus ipilimumab infusion in mesothelioma patients.

- To identify the recommended phase 2 dose (RP2D) of intratumorally administered LMB-100 + ipilimumab in patients with treatment refractory advanced malignant mesothelioma

10.1.2 10.1.2 Secondary Objectives

- To preliminarily determine the objective response rate of combination therapy with intratumorally administered LMB-100 plus ipilimumab.
- To determine the duration of response (DOR), progression free survival (PFS) and overall survival (OS) with intratumorally administered LMB-100 plus ipilimumab.

10.2 SAMPLE SIZE DETERMINATION

The primary objective of this trial is to determine the safety and tolerability of intratumor administered LMB-100 plus ipilimumab. An important secondary objective is to determine in a preliminary fashion if this combination, at two dose levels (including DL-1 and DL1, or DL1 and DL2) treated combined, is associated with an objective response rate (ORR) which may potentially exceed that of ipilimumab alone in patients who have mesothelioma that has failed standard therapies (10% response rate). Other secondary objectives are to evaluate duration of response (DOR), overall survival (OS), and progression-free survival (PFS).

Using a 3+3 design based on safety, consisting of a two dose-levels, patients with pleural or peritoneal mesothelioma will be enrolled onto the study. Thus, up to 6 patients per dose level may enroll during dose escalation (maximum of 12 patients to determine the MTD). A dose level -1 will also be available if needed. The two participants enrolled while the study design called for 2 cycles of LMB-100 will also be included in the evaluable participants as they will be included in the toxicity evaluation and exploratory analyses. In order to account for potential screen failures (four) and inevaluable subjects (two) the accrual ceiling will be set at 20.

It is expected that all 14 evaluable patients may enroll onto this trial during one to two years.

10.3 POPULATIONS FOR ANALYSES

Modified intention to treat: any subjects who enroll onto the trial, provide consent, and receive at least one dose of LMB-100 will be evaluated for safety.

Patients who receive at least one dose of both LMB-100 and ipilimumab at the MTD or highest safe dose will be evaluable for clinical response.

10.4 STATISTICAL ANALYSIS

10.4.1 General Approach

The safety in the patients enrolled during dose escalation will be evaluated by reporting the grade and type of toxicity at each dose level.

The fraction of all patients evaluated at the MTD who experience a response will be reported separately along with confidence intervals.

10.4.2 Analysis of the Primary Endpoints

The safety in the dose escalation portion of the trial will be evaluated by reporting the grade and type of toxicity at each dose level evaluated. The feasibility of administering LMB-100 will be assessed by determining the fraction of patients at a dose level who did not experience a DLT.

10.4.3 Analysis of the Secondary Endpoints

Clinical responses will be analyzed by reporting the fraction of evaluable patients treated at the highest safe level who experience a response, along with a 95% two-sided confidence interval. In addition, an overall response rate based on the results from all patients treated at the two evaluated dose levels will be determined and reported along with a 95% confidence interval. The response results from the combined dose level evaluation may be used to guide whether the combination will be investigated further in subsequent studies with caveats about the limited numbers of patients in the evaluations.

To measure overall and progression-free survival, and duration of response (beginning at the date response is noted), the Kaplan-Meier method will be used, and the median DOR, OS and PFS will be reported along with a 95% two-sided confidence interval. This will be reported separately for all patients enrolled, and for the evaluable patients treated at the MTD or highest safe dose with caveats about the limited numbers of patients in the evaluations.

10.4.4 Safety Analyses

The fraction of patients who experience a toxicity, by grade and type of toxicity, will be tabulated, by dose level.

10.4.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported for all patients on the trial.

10.4.6 Planned Interim Analyses

Only those needed to establish adequate safety to continue to the next dose level will be performed.

10.4.7 Halting Rules

For safety reasons, the enrollment will be temporarily halted until an expedited safety report has been evaluated by the investigators, IND sponsor, submitted to the FDA and the decision to continue trial has been approved by the Sponsor review for the following event attributable to treatment regimen occurring within 30 days of receiving LMB-100:

- One occurrence of Grade 5 toxicity

10.4.8 Sub-Group Analyses

None.

10.4.9 Tabulation of individual Participant Data

None.

10.4.10 Exploratory Analyses

The following are the exploratory objectives and the methods of analysis

- To assess the effect of ipilimumab on pharmacokinetics and antidrug antibody development against LMB-100, descriptive analyses will be performed.
- To establish the association of response with tumor mesothelin expression, the levels will be compared between responders and non-responders using an appropriate exact two-group

non-parametric test, such as the Wilcoxon rank sum test. The analyses will be done for all patients and separately for the patients treated at the MTD or highest dose level.

- To evaluate changes in the tumor microenvironment as well as plasma cytokine release following treatment with intratumorally administered LMB-100 and ipilimumab. Any analyses done for this endpoint will be done using descriptive methods only.
- To evaluate the utility of serum mesothelin and megakaryocyte potentiating factor (MPF) as biomarkers of tumor response, the levels will be compared between responders and non-responders using an appropriate exact two-group non-parametric test, such as the Wilcoxon rank sum test. The analyses will be done for all patients and separately for the patients treated at the MTD or highest dose level.

Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 HUMAN SUBJECTS PROTECTIONS

11.1 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). LMB-100 has shown synergy in laboratory models with inhibitors of CTLA-4. Ipilimumab is an anti-CTLA-4 inhibitor. The rationale to evaluate LMB-100 with ipilimumab in advanced/metastatic mesothelioma is to determine the effect of the addition of ipilimumab to intratumoral LMB-100 therapy. All patients meeting the criteria listed in section 2.1 are eligible for enrollment.

11.2 PARTICIPATION OF CHILDREN

There are no dosing or adverse event data are currently available on the use of LMB-100 with ipilimumab in patients <18 years of age; therefore, children are excluded from this study.

11.3 PARTICIPATION OF SUBJECTS UNABLE TO CONSENT

Adults unable to provide consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. For subjects that lose the ability to consent due to tumor progression, they will be taken off treatment but will have the opportunity to remain on study in long term follow-up for collection of outcomes for overall survival. If the reason for incapacity is due to a reason other than definitive disease progression and they are felt to be responding to the treatment under study in the opinion of the investigator, they will have the opportunity to continue this therapy.

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. Please see section for the consent procedure. 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.

11.4 RISK/BENEFIT ASSESSMENT

11.4.1 Known Potential Risks

11.4.1.1 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 agents as discussed in section 13.

11.4.1.2 Study Drug Administration

The LMB-100 on this study with be administered intratumorally. The risks of intratumor injection include acute injection site pain and fever and injection site erythema. In rare instances, lung injury or bowel perforation may occur.

11.4.1.3 Blood Collection

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. The maximum amount of blood required at a single timepoint is 69.5 mL with a maximum of 365.5 mL required over a given 8 week period.

11.4.1.4 Biopsy Collection

The risks of the biopsies collected at screening (if needed) and with each dose of LMB-100 (if feasible), before the first dose of ipilimumab and after the last dose of ipilimumab include pain, bleeding and infection at the biopsy site.

11.4.1.5 Risks of Anesthesia

As noted, anesthesia will be provided to control pain/discomfort while injecting the tumor. As cycle 1 biopsies are collected in conjunction with tumor injection, pain management for the biopsies may include all three types listed below. After cycle 1, general anesthesia will not be used for the collection of the research biopsy, but the other two options remain.

11.4.1.5.1 Local anesthesia

Participants receiving local anesthesia may experience an allergic reaction to the local anesthetic.

11.4.1.5.2 Conscious sedation

Potential side effects of conscious sedation include headache, nausea, slow reflexes, low blood pressure, amnesia and drowsiness. These side effects usually go away quickly.

11.4.1.5.3 General anesthesia

Risks of general anesthesia include temporary confusion and memory loss, although this is more common in the elderly, dizziness, difficulty passing urine, muscle aches, itching, bruising or

soreness from the IV drip, nausea and vomiting, shivering and feeling cold, sore throat due to the breathing tube.

11.4.1.6 Imaging

In addition to the radiation risks discussed below, scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, experience a metallic taste, headache, difficulty breathing, hypotension, increased heartrate and swelling. Furthermore, the IV catheter used to administer the contrast may cause bleeding, infection or inflammation of the skin and vein with pain and swelling. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage. MRIs include the additional risk of damage to hearing.

11.4.1.7 Radiation

The study will involve radiation from the following sources:

- Up to 5 CT scans for use in intralesional injection and collection of biopsies
- Up to 9 FDG-PET scans per year for disease assessment
- Up to 9 CT scans per year for disease assessment

Subjects in this study may be exposed to approximately 19.3 rem. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 1.9 out of 100 (1.9%) and of getting a fatal cancer is 1 out of 100 (1%).

11.4.2 Known Potential Benefits

Although it is anticipated that the combination of intrapleural LMB-100 and ipilimumab will induce an immune response against tumors in mesothelioma, at this time, there are no known direct benefits of the planned combination.

11.4.3 Assessment of Potential Risks and Benefits

Patients malignant 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. Laboratory studies have further demonstrated synergy with CTLA-4 inhibitors. Therefore, LMB-100 + ipilimumab 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 outweigh the risks associated with the proposed entry-into-human trial with LMB-100 + ipilimumab

11.5 Consent Process and Documentation

The informed consent document will be provided in physical or electronic format to the participant or consent designee(s) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family

members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with local policy, including HRPP policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

11.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section 11.3, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in section 11.5.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to IND sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

12.2 Quality Assurance and Quality Control

Each 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.

12.3 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 National Cancer Institute 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.

12.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. 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 the 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 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 the clinical sites will be secured and password protected. At the end of the study, all study databases will be archived at the NIH Clinical Center.

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.

13 PHARMACEUTICAL INFORMATION

13.1 LMB-100 (IND # 152907)

13.1.1 Source /Acquisition and Accountability

LMB-100 (investigational agent) was transferred to the NIH CC Pharmacy by Selecta Biosciences, the drug manufacturer. For this trial, the drug will be supplied by the NIH CC Pharmacy.

13.1.2 Toxicity

Information in this section is based on clinical studies of LMB-100.

Toxicities Observed in Participants Receiving LMB-100 Alone or in Combination

System Organ Class	NCI Common Terminology Criteria for Adverse Events (CTCAE) Term	# of Subjects	% of participants
Blood and Lymphatic system Disorders	Anemia	37	29.84
Cardiac Disorders	Atrial fibrillation	7	5.65
	Atrial flutter	1	0.81
	Chest pain - cardiac	1	0.81
	Myocarditis	1	0.81

System Organ Class	NCI Common Terminology Criteria for Adverse Events (CTCAE) Term	# of Subjects	% of participants
	Palpitations	4	3.23
	Pericardial effusion	8	6.45
	Pericardial tamponade	3	2.42
	Pericarditis	3	2.42
	Sinus tachycardia	25	20.16
Ear and Labyrinth Disorders	External ear pain	1	0.81
Endocrine Disorders	Hypothyroidism	1	0.81
Eye Disorders	Eye disorders - Other, specify	1	0.81
Gastrointestinal Disorders	Abdominal distension	2	1.61
	Abdominal pain	9	7.26
	Ascites	1	0.81
	Constipation	5	4.03
	Diarrhea	5	4.03
	Dry mouth	1	0.81
	Dyspepsia	4	3.23
	Nausea	29	23.39
	Upper gastrointestinal hemorrhage	1	0.81
	Vomiting	14	11.29
General Disorders and Administration Site Conditions	Chills	5	4.03
	Edema face	7	5.65
	Edema limbs	39	31.45
	Fatigue	54	43.55
	Fever	25	20.16
	General disorders and administration site conditions - Other, specify	1	0.81
	Generalized edema	1	0.81
	Irritability	1	0.81
	Localized edema	22	17.74
	Malaise	1	0.81
	Non-cardiac chest pain	6	4.84
	Pain	10	8.06
Immune System Disorders	Allergic reaction	1	0.81
	Immune system disorders - Other, specify	1	0.81
Infections and Infestations	Herpes simplex reactivation	1	0.81
	Infections and infestations - Other, specify	1	0.81
	Lung infection	1	0.81

System Organ Class	NCI Common Terminology Criteria for Adverse Events (CTCAE) Term	# of Subjects	% of participants
Injury, Poisoning and Procedural Complications	Fall	1	0.81
	Infusion related reaction	14	11.29
Investigations	Alanine aminotransferase increased	31	25
	Alkaline phosphatase increased	11	8.87
	Aspartate aminotransferase increased	47	37.9
	Cardiac troponin I increased	5	4.03
	CPK increased	5	4.03
	Creatinine increased	33	26.61
	Ejection fraction decreased	1	0.81
	Investigations - Other, specify	1	0.81
	Lymphocyte count decreased	54	43.55
	Neutrophil count decreased	9	7.26
	Platelet count decreased	14	11.29
	Thyroid stimulating hormone increased	3	2.42
	Urine output decreased	1	0.81
	Weight gain	42	33.87
	Weight loss	3	2.42
	White blood cell decreased	21	16.94
Metabolism and Nutrition Disorders	Anorexia	23	18.55
	Dehydration	5	4.03
	Hypercalcemia	1	0.81
	Hyperglycemia	1	0.81
	Hypernatremia	2	1.61
	Hyperuricemia	2	1.61
	Hypoalbuminemia	99	79.84
	Hypocalcemia	7	5.65
	Hypokalemia	8	6.45
	Hypomagnesemia	2	1.61
	Hyponatremia	49	39.52
	Hypophosphatemia	5	4.03
Musculoskeletal and Connective Tissue Disorders	Arthralgia	6	4.84
	Arthralgia	6	4.84
	Back pain	4	3.23
	Flank pain	3	2.42
	Generalized muscle weakness	3	2.42
	Myalgia	33	26.61

System Organ Class	NCI Common Terminology Criteria for Adverse Events (CTCAE) Term	# of Subjects	% of participants
	Neck pain	1	0.81
	Pain in extremity	2	1.61
	Rhabdomyolysis	1	0.81
Neoplasms Benign, Malignant and Unspecified (Incl Cysts And Polyps)	Tumor pain	3	2.42
Nervous System Disorder	Confusion	2	1.61
	Dizziness	12	9.68
	Dysgeusia	1	0.81
	Headache	8	6.45
	Lethargy	1	0.81
	Peripheral motor neuropathy	1	0.81
	Peripheral sensory neuropathy	8	6.45
	Presyncope	1	0.81
	Somnolence	1	0.81
	Syncope	1	0.81
	Tremor	1	0.81
Psychiatric Disorders	Insomnia	2	1.61
Renal and Urinary Disorders	Acute kidney injury	5	4.03
	Proteinuria	9	7.26
Reproductive System and Breast Disorders	Pelvic pain	1	0.81
Respiratory, Thoracic and Mediastinal Disorders	Allergic rhinitis	1	0.81
	Cough	2	1.61
	Dyspnea	27	21.77
	Hypoxia	5	4.03
	Pleural effusion	7	5.65
	Pleuritic pain	5	4.03
	Pneumonitis	1	0.81
	Productive cough	1	0.81
	Pulmonary edema	1	0.81
	Respiratory failure	1	0.81
	Sore throat	2	1.61
	Upper respiratory infection	1	0.81
Skin and Subcutaneous Tissue Disorders	Alopecia	10	8.06
	Dry skin	1	0.81
	Periorbital edema	2	1.61
	Pruritus	3	2.42
	Rash maculo-papular	4	3.23

System Organ Class	NCI Common Terminology Criteria for Adverse Events (CTCAE) Term	# of Subjects	% of participants
	Skin and subcutaneous tissue disorders - Other, specify	1	0.81
Vascular Disorders	Capillary leak syndrome	11	8.87
	Flushing	2	1.61
	Hypertension	4	3.23
	Hypotension	34	27.42
	Thromboembolic event	1	0.81

13.1.2.1 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[48] .

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.

13.1.3 Formulation

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.

13.1.4 Preparation

Warm vials in the hand for 10 to 20 seconds. Inspect vials visually. Do not use if material appears turbid. Do not shake; proteins can foam and may denature. Do not filter.

Dose Level	0.2 % HSA in 0.9% NaCl	LMB-100
DL -1	3800 mcL	200 mcg (200 mcL from single use vial)
DL 1	3600 mcL	400 mcg (400 mcL from single use vial)
DL 2	2800 mcL	1200 mcg (1200 mcL from single use vial)

Dilute the required amount of LMB-100 with 0.2% human derived HSA in 0.9% sodium chloride to a total volume of 4 mL in single use syringe. Constituents should be added in the following order:

1. Human Serum Albumin (final concentration 0.2%)
2. 0.9% sodium chloride
3. LMB-100

Agitate gently to disperse.

13.1.5 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.

13.1.6 Administration procedures

Please refer to section 3.3.1.

13.1.7 Incompatibilities

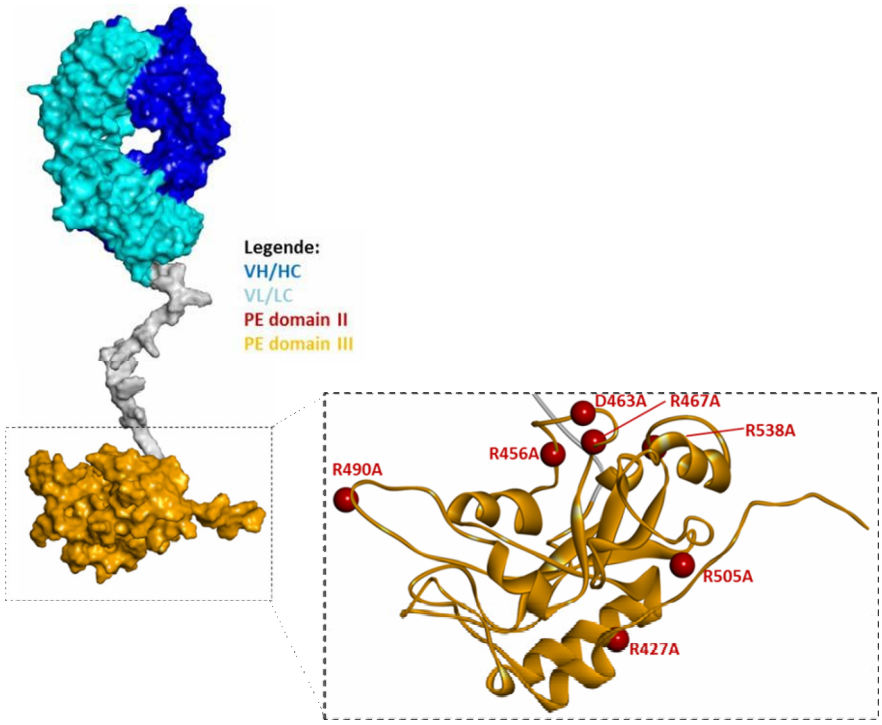
Pharmacodynamic drug interaction studies have not been conducted. LMB-100 is contraindicated in subjects with a history of severe allergic anaphylactic reactions to humanized, chimeric or mouse peptides/antibodies or to any components of the product.

13.1.7.1 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.

13.1.7.2 Molecular Weight: approximately 73 kDa

13.1.7.3 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.

13.2 IPILIMUMAB

13.2.1 Source /Acquisition and Accountability

Ipilimumab will be purchased from commercial sources by the NIH CC Pharmacy. The pharmacy will distribute the drug per protocol. Disposal will be per NIH CC SOPs.

13.2.2 Toxicity

Please refer to package insert and informed consent document.

13.2.3 Formulation and preparation

Please refer to package insert.

13.2.4 Stability and Storage

Please refer to package insert.

13.2.5 Administration procedures

Please refer to section 3.3.2.

13.2.6 Incompatibilities

Please refer to package insert.

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15 APPENDICES

15.1 APPENDIX A. PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.