Abbreviated Title: LMB-100 plus SEL-110
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Title: A Phase I Study of the Mesothelin-Targeted Immunotoxin LMB-100 in Combination with SEL-110 in Subjects with Malignant Pleural or Peritoneal Mesothelioma

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Investigational Agents:

Drug Name:	LMB-100 (formerly RO6927005)	SEL-110
IND Number:	136483	136483
Sponsor:	Center for Cancer Research, NCI, NIH	Center for Cancer Research, NCI, NIH
Manufacturer:	F. Hoffmann-La Roche	Selecta Biosciences, Inc.

Commercial Agents: None

PRÉCIS

Background:

- LMB-100 and a closely related immunotoxin also targeting mesothelin have been studied in previous Phase 1 clinical studies for mesothelioma and pancreatic cancer.
- Results from these studies showed that the majority of patients formed anti-drug-antibodies (ADAs) that neutralized subsequent injection of the product making it ineffective.
- In a small subset of patients that did not form ADAs to the product, good response and regression of tumors was seen.
- In a different application SEL-110, a biodegradable nanoparticle containing rapamycin, has been shown in clinical trials to prevent the formation of ADAs to an immunogenic enzyme when co-administered. Preclinical data show that SEL-110 also prevents the formation of ADAs to LMB-100.
- This clinical trial will investigate whether SEL-110 when administered with LMB-100 is able to prevent the formation of ADAs and thus allow patients to receive multiple, effective injections of LMB-100.

Objectives:

- The primary objective of the study is to assess the safety and tolerability of LMB-100 in combination with SEL-110.

Eligibility:

Primary Inclusion Criteria

- ≥ 18 years of age
- Histologically confirmed epithelial or biphasic pleural or peritoneal mesothelioma not amenable to potentially curative surgical resection.
- Patients must have measurable disease per RECIST 1.1.
- Patients must have had at least one prior chemotherapy regimen that includes pemetrexed and cisplatin or carboplatin. There is no limit to the number of prior chemotherapy regimens received.
- Patients for whom no standard curative therapy exists

Primary Exclusion Criteria:

- Known or clinically suspected CNS primary tumors or metastases including leptomeningeal metastases.
- Evidence of significant, uncontrolled concomitant diseases which could affect compliance with the protocol or interpretation of results.
- Evidence of active or uncontrolled infections.

- Live attenuated vaccinations 14 days prior to treatment
- Pregnant women are excluded from this study

Design:

- This is a Phase I, single center, dose escalation study of LMB-100 in combination with SEL-110
- Patients will receive the combination using a dose escalation scheme in which different doses of LMB100 and SEL-110 will be evaluated.
- Patients will receive 4 cycles of LMB-100 with SEL-110. A cycle will consist of i.v. infusion of SEL-110 on Day 1 of the cycle followed immediately by an i.v. infusion of LMB-100, then on Days 3 and 5 of the cycle patients will receive an i.v. infusion of LMB-100 only. Treatment cycles will be separated by 21 days.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- The primary objective of the study is to assess the safety and tolerability of LMB-100 in combination with SEL-110

1.1.2 Secondary Objectives

- To determine if the use of SEL-110 along with LMB-100 for treatment of mesothelioma is associated with decreased formation of antibodies to LMB-100
- To define the effect of SEL-110 on the pharmacokinetics characteristics of LMB-100
- To assess the objective response rate (complete response + partial response) of SEL-110 in combination with LMB-100

1.1.3 Exploratory Objectives

- To establish the correlation of response to treatment with tumor mesothelin expression
- To investigate the potential of soluble mesothelin levels and levels of megakaryocyte potentiating factor to predict any therapeutic response
- To determine the frequency anti-PEG antibody formation
- To identify the mechanism for Pseudomonas exotoxin-mediated capillary leak syndrome
- To evaluate changes in the tumor microenvironment following treatment with LMB-100 + SEL-110

1.2 BACKGROUND AND RATIONALE

1.2.1 Background on Mesothelioma

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

Peritoneal mesothelioma represents about one-fifth to one-third of all forms of mesothelioma; there are approximately 400 new cases in the United States each year.(3) Cytoreductive surgery and hyperthermic perioperative chemotherapy is the accepted initial management for suitable

patients with peritoneal mesothelioma.(4-7) Peritoneal mesothelioma patients with surgically unresectable disease or whose medical co-morbidities preclude surgery are considered for palliative systemic therapy. Due to its relatively low incidence and inherent difficulties of radiologic assessment, few studies of systemic therapy have been conducted. Treatment recommendations are often extrapolated from pleural mesothelioma and outcomes are poor.

1.2.2 Mesothelin as a target for cancer therapy

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

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

1.2.2.1 Recombinant Immunotoxins (RITs)

1.2.2.1.1 Mechanism and structure of RITs

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

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

by the US Food and Drug Administration (FDA); a fusion protein of diphtheria toxin with interleukin-2 that is clinically used for intravenous treatment of cutaneous T cell lymphoma.

1.2.2.1.2 SS1P - a recombinant immunotoxin targeting mesothelin

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

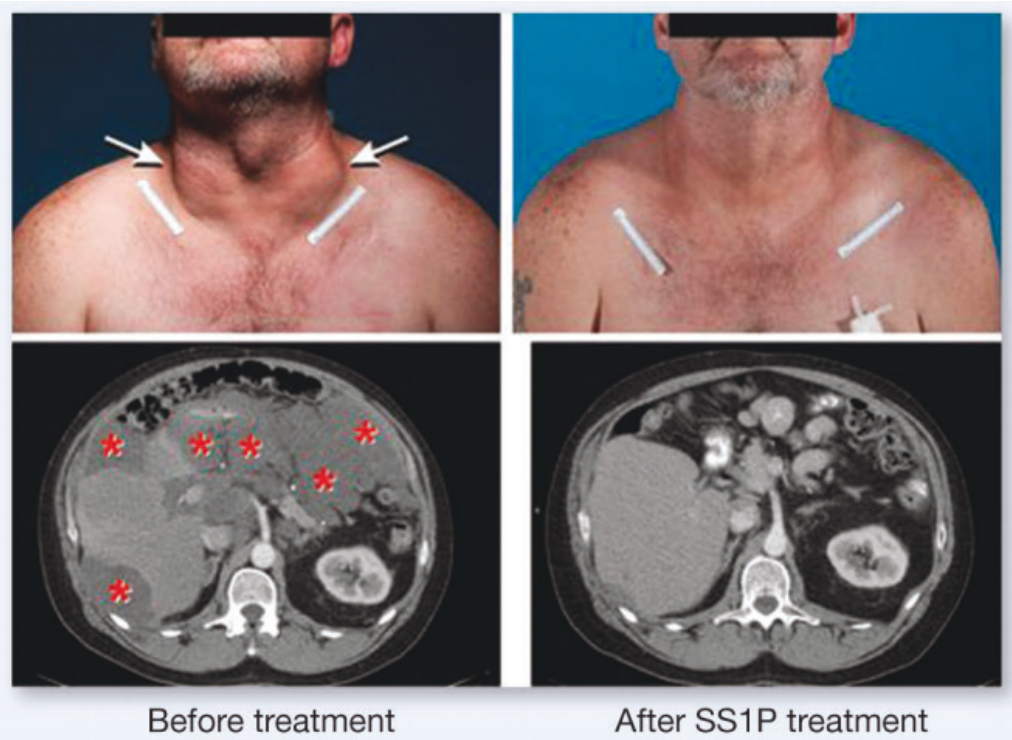
These early trials demonstrated that the efficacy of SS1P is limited by its immunogenicity. Anti-SS1P neutralizing antibodies (Nabs) formed after one cycle in ~90% of patients preventing effective drug exposure in subsequent cycle.(12, 14) Development of human antibody response to immunotoxins in patients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

This strategy of using pentostatin and cyclophosphamide to prevent human antibody response to SS1P was evaluated in a pilot study of heavily pre-treated chemotherapy refractory mesothelioma patients. Eleven patients were enrolled in the study and received pentostatin and cyclophosphamide prior to SS1P administration. This regimen was well tolerated and no patient developed opportunistic infections. As predicted this regimen decreased the development of human antibodies to SS1P; only two of ten patients developed antibodies after cycle 1 of therapy, which was significantly better than in prior clinical trials where about 90% of patients developed antibodies after one cycle of therapy.(19) Remarkably, three of the ten evaluable patients with extensive tumor burden had durable partial response and two of these patients had complete metabolic response by PET scan. All three patients were alive after 27, 25 and 22 months of starting therapy. In addition, two patients who did not initially respond to SS1P had a dramatic anti-tumor response when treated with chemotherapy to which they had not previously responded. SS1P plus pentostatin/cyclophosphamide regimen is currently being evaluated in more patients with mesothelioma. Major tumor shrinkage in the neck and abdomen of a patient with metastatic peritoneal mesothelioma treated with SS1P in combination with pentostatin and cyclophosphamide is shown in Figure 1.

These results provide a proof of principle that RITs can have meaningful clinical efficacy in patients with advanced solid tumors. A new generation of immunotoxin molecules with reduced

immunogenicity and non-specific toxicity has been developed through protein engineering techniques.

Figure 1. Representative response to SS1P in a mesothelioma patient



1.2.3 LMB-100

1.2.3.1 Rationale for the development of LMB-100

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

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,(20) but has also resulted in reduced incidents of VLS in animal models of VLS.(21)

1.2.3.2 Development of LMB-100:

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

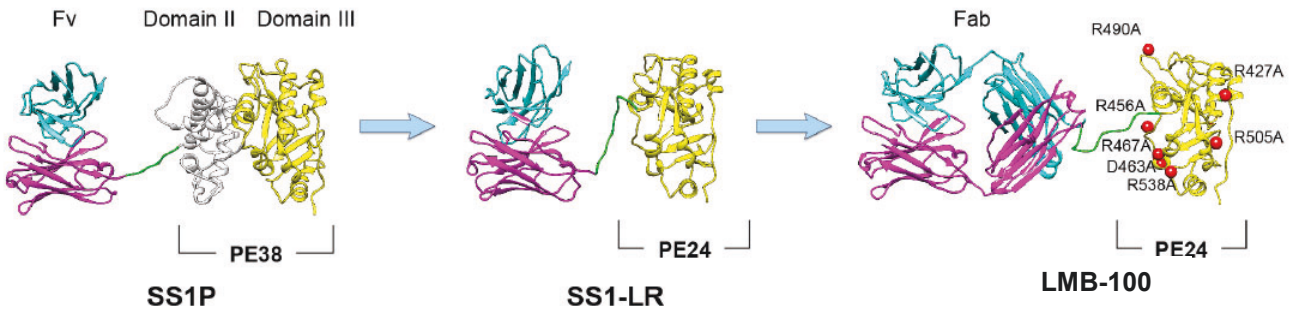


Figure 2. Structural models of SS1P and its de-immunized variants SS1P-LR and LMB-100 are shown.

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

1.2.3.3 Nonclinical Studies

1.2.3.3.1 Nonclinical Pharmacology

In vitro LMB-100 inhibited viability of a variety of mesothelin-positive cancer cell lines at effective concentrations typically around 14 pM (~1 ng/mL). The cytotoxic potency of LMB-100 varied between 0.35 ng/mL in primary mesothelioma cells (RH21) and 15.7 ng/mL in an adenosquamous lung carcinoma cell line (H596). Binding studies showed that while the Fab fragment did not bind to mouse or rat mesothelin, the binding affinities to cynomolgus and human mesothelin were identical. In agreement with this, LMB-100 induced apoptosis in mesothelin-positive primary cynomolgus pericardial cells and significantly impaired viability of HEK293 cells

transfected with human mesothelin, but not of rat mesothelin transfected or untransfected HEK293 cells. In addition, control experiments showed that free PE24 was 100–1000 fold less potent on mesothelin-positive target cell lines, confirming low cytotoxic potential of PE24 lacking a targeting moiety.

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

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

1.2.3.3.2 *Pharmacokinetics in Animals*

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

The relationship between systemic drug exposure and anti-tumor activity of LMB-100 was investigated on human lung cancer NCI-H596 xenograft growth in female SCID beige mice. Free

and total drug profiles were similar in mice. Modeling estimated a plasma concentration of 6800 ng/mL (± 36%) to trigger a half maximal rate of tumor regression. Concentrations of LMB-100 above this level resulted in potent tumor regression after dosing. Normalized for exposure, SS1P was found to be ~3-fold more potent than LMB-100 in terms of tumor growth inhibition.

1.2.3.3.3 *Toxicology and Safety Pharmacology*

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

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

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

The potential of LMB-100 to induce off-target vascular leak in lungs was assessed in female Wistar rats. Mild perivascular edema was reported microscopically, but did not correlate with macroscopic or serum chemistry findings consistent with VLS. Ultrasound evaluation in the NHP

GLP study revealed minimal accumulation of pericardial fluid with limited biological significance at the highest dose of 1 mg/kg. No appreciable accumulation of pleural fluid was observed at necropsy.

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

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

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

A total of 15 participants were enrolled onto the study before termination. Median age of participants was 60.8 years and 53.3% were female. All participants had received prior anti-cancer therapy for their tumors. Enrolled participants had advanced mesothelioma (7), ovarian cancer (3), pancreatic cancer (3), and gastroesophageal cancer (2). Tumors from 13 of the 15 participants treated had moderately to strongly positive MSLN expression as measured by central IHC analysis.

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

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

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

Table 1. LMB-100 Dose escalation study- NCT02317419		
Dose (mcg/kg)	No. of patients	Pts with DLT
DLTs were vascular leak syndrome and proteinuria NE, Study terminated before DLT assessment period was complete and patients only received single dose of LMB-100		

1.2.3.4.1 LMB-100 Adverse Events

Overall, 14 participants (93.3%) experienced at least one AE. The most common AEs were hypoalbuminemia (60.0%), fatigue (53.3%), peripheral edema (53.3%), nausea (46.7%), pyrexia (40.0%), decreased appetite (33.3%), dyspnea (33.3%), and myalgia (33.3%). SAEs included vascular leak syndrome, pyrexia, atrial flutter, infusion related reaction, arthritis, glomerulonephritis minimal lesion and dyspnea. No participants experienced an AE that led to withdrawal of study treatment. Four participants experienced a total of 8 infusion-related reactions that were independent of drug dose level. These AEs were non-serious and resolved within approximately 1 hour of onset. Pre-medication for infusion reaction was administered to these participants prior to subsequent doses of LMB-100. Two suspected Type III hypersensitivity reactions were observed. These consisted of arthritis (1 patient) and rash with fever (1 patient), both of which were fully reversible. When other AEs attributed to the study drug are presented by dose level of drug, it becomes clear that toxicity was strongly associated with the 250 mcg/kg dose level at which DLT was reached. Two of four patients treated at 250 mcg/kg experienced serious VLS which manifested with hypotension, respiratory compromise, serosal membrane reaction and hyponatremia as well as the hypoalbuminemia and edema that can be seen with mild VLS. Other symptoms associated with the DLT dose were fatigue, nausea, vomiting, decreased appetite and mild elevation of transaminases. Table 2 summarizes any adverse event related to LMB-100 while as Table 3 summarizes grade 3 or 4 toxicity related to LMB-100. As shown in Table 3 most grade 3 or 4 toxicity were seen in patients treated at 250 mcg/kg dose level. However, patients receiving LMB-100 doses less than 250 mcg/kg had mainly grade 1-2 adverse events except 1 patient having grade 3 arthritis and 1 patient with grade 3 anemia.

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

Dose (mcg/kg)	45	65	100	170	250	**ALL**
# of patients treated	1	1	3	4	4	**13**
Vascular Leak	-	-	1	1	2	**4**
grade 3 or 4 vascular leak	-	-	-	-	1	**1**
hypotension	-	-	-	-	2	**2**
hypoalbuminemia	-	1	3	2	3	**9**
peripheral edema	1	1	3	2	4	**11**
facial edema	-	1	2	-	1	**4**
weight gain	-	-	1	-	1	**2**
hyponatremia	-	-	-	-	3	**3**

Dose (mcg/kg)	45	65	100	170	250	**ALL**
# of patients treated	1	1	3	4	4	**13**
hypophosphatemia	-	-	-	-	1	**1**
dyspnea	-	1	-	-	2	**3**
Infusion related reaction	-	-	2	-	2	**4**
Constitutional						
fatigue	-	-	-	1	4	**5**
asthenia	-	-	3	-	-	**3**
fever	1	-	2	1	2	**6**
Musculoskeletal						
myalgia	-	-	1	1	2	**4**
arthralgia	1	-	-	-	1	**2**
arthritis	-	-	1	-	-	**1**
muscle spasm	-	-	-	1	-	**1**
Cardiac						
pericardial effusion	-	-	-	-	1	**1**
Atrial flutter	-	-	-	1	-	**1**
Renal Disorders						
glomerulonephritis minimal	-	-	-	1	-	**1**
proteinuria	-	-	-	1	1	**2**
Creatinine increase	-	-	-	-	2	**2**
Gastrointestinal						
decreased appetite	-	1	-	1	2	**4**
nausea	1	-	1	1	4	**7**
abdominal pain	-	1	-	1	1	**3**
diarrhea	1	-	1	-	-	**2**
vomiting	-	-	-	-	2	**2**
abdominal distension	-	1	-	-	-	**1**
constipation	-	-	-	1	-	**1**
dyspepsia	-	-	-	1	-	**1**
AST increase	-	-	-	-	2	**2**
Hematologic						
anemia	-	-	-	1	-	**1**
decreased lymphocytes	-	-	-	-	1	**1**
Total Grade 3 or greater	-	-	2	1	2	**5**

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

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

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

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

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

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

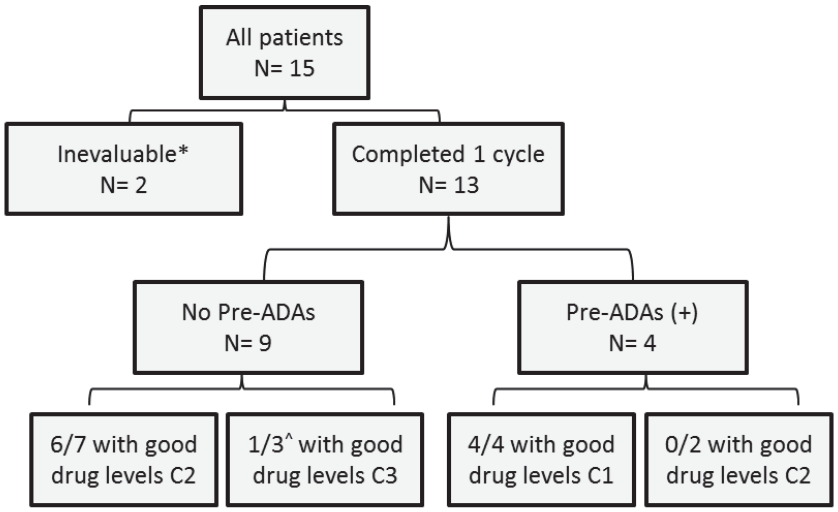
Twelve participants were evaluable for efficacy. The best confirmed overall response was stable disease in 3 participants. A Roche-developed ELISA test was used to retrospectively assess anti-drug antibody (ADA) titers. Four of 13 patients had detectable ADAs at study enrollment while the remaining participants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

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

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

to determine what percent of patients will achieve good blood levels during cycle 3 and beyond.

Figure 3. Effect of anti-drug antibodies (ADA) on the ability to achieve good LMB-100 blood levels in patients treated with LMB-100.



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

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

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

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

Patient	Dose (mcg/kg)	Cycle 1		Cycle 2			Cycle 3		
		ADA (Day1)	C_{max} (ng/ml)	ADA (Day1)	Cmax (ng/ml)	dC1/dC (%)	ADA (Day1)	C_{max} (ng/ml)	dC1/dC (%)

* Patient's treatment stopped due to study closure

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

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

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

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

* Patient's treatment stopped due to study closure

1.2.3.5 Clinical testing of single agent LMB-100 at NCI (Study – 16C0127 - ongoing)

As of July 12, 2017, ten patients have been enrolled on the study. Out of these 10 patients 7 had peritoneal mesothelioma and 3 pleural mesothelioma; 6 female and 4 male and median age was 62 years old. The first 3 patients were treated at dose level 1 i.e. 170 mcg/kg with patient #1 initiating treatment on July 28, 2016. All three patients at this dose level during cycle 1 had grade 1 or 2 increase in serum creatinine). Since increase in serum creatinine was a common toxicity pattern at this dose level it was defined as DLT per protocol established criteria. The protocol was subsequently amended to allow the treating these three patients at dose level -1 (140 mcg/kg) during cycle 2-4 though the protocol prior to the amendment would have allowed retreatment at 170 mcg/kg as long as they met inclusion criteria for the study especially adequate renal function, defined in the protocol as creatinine clearance (by Cockcroft Gault formula) $\geq$50 mL/min. However, we felt it would be safer to re-treat these patients at dose level-1 instead of dose level 1 and adjusted the dose modification section of the protocol accordingly.

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

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

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

Out of 10 patients who have been evaluated for tumor response after cycle 2, 9 had stable disease and 1 had progressive disease.

Between July 2016 and November 2016, 10 patients were treated with LMB-100 monotherapy. Details of the treatment related adverse events (highest grade per patient) are provided in Table 6. In summary, there were no treatment-related grade 4 or 5 events. One patient had grade 3 hyponatremia. Most common grade 2 adverse events included hypoalbuminemia, infusion reactions, anemia, and lymphedema. Best response was stable disease in 9 patients and progressive disease in one. Of 9 patients who had stable disease, 2 patients progressed at the end of that course of therapy (4 cycles). Duration of stable disease in the remaining patients ranged between 2 months to ongoing at 8 months.

Table 6. LMB-100 monotherapy treatment related adverse events highest grade per patient

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Alanine aminotransferase increased	5				
Anemia		3			
Anorexia	3				
Arthralgia	2				
Aspartate aminotransferase increased	8				
Atrial flutter		2			
Chills	1	1			
Creatinine increased	4	2			
Dizziness	1				
Dyspepsia	2	1			
Dyspnea	5				
Edema face	2				
Edema limbs	6	3			
Fatigue	8				
Fever	3	1			
Flushing	2				

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Headache	1				
Hypertension	2				
Hyperuricemia	1				
Hypoalbuminemia	3	7			
Hyponatremia	2		1		
Hypotension	3				
Infusion related reaction		5			
Myalgia	9				
Nausea	6	1			
Pain	3				
Palpitations	2				
Pelvic pain	1				
Periorbital edema	2				
Platelet count decreased	1	1			
Pleuritic pain	1				
Sinus tachycardia	6				
Tumor pain	2	1			
Vomiting	2				
Weight gain	4	2			

1.2.3.5.1 Pharmacokinetics of LMB-100:

Free LMB-100 plasma concentrations were measured with a validated ELISA with a lower limit of quantification of 2.1 ng/mL. Doses ranged from 140 mcg/kg – 170 mcg/kg. Samples for pharmacokinetic (PK) analysis were obtained from patients at pre-dose, end of infusion (EOI; 30-min post start), and 1 hour, 2 hours, 3 hours, 4 hours and 6 hours post EOI. Concentration data for each dose was plotted over time to assess the impact of increasing anti-drug antibodies (ADAs) that are generated in response to LMB-100 exposure. PK analysis for the 10 patients treated with single agent LMB-100 is shown in Table 7 below. Measured LMB-100 plasma concentrations were consistent during the first week of treatment, with a near dose-proportional increase in C_{MAX} from 140 mcg/kg to 170 mcg/kg. However, the suspected generation of ADAs greatly reduced LMB-100 exposure by cycle 2.

As shown in Table 7, all 10 patients had good LMB-100 blood levels during cycle 1. However, only 5 of 10 patients had good blood levels during cycle 2. None of the 8 patients who got cycle 3 and 4 of LMB-100 had good LMB-100 blood levels during cycle 3 or 4. We defined good blood levels, as LMB-100 Cmax concentration of >200 ng/mL at end of infusion on day 1 for each cycle. Although an arbitrary cut-off, it represents blood levels more than 50 to 100 fold greater than those required to kill mesothelin expressing cells in-vitro. However, more patients will need to be treated to truly correlate LMB-100 blood levels with therapeutic benefit and is one of the objectives of this protocol.

Table 7. Decrease in LMB-100 Exposure Over Time

Patient	C1D1		C2D1		C3D1		C4D1	
	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)	Dose (µg/kg)	Cmax (ng/mL)
1	170	1991	140	1091	140	21	140	21
2	170	2286	140	297	140	70	140	21
3	170	2760	140	4.4	140	BQL	140	BQL
4	140	1054	140	681	140	BQL	140	BQL
5	140	1124	140	BQL	140	–	140	–
6	140	2584	140	1099	140	BQL	140	BQL
7	140	3118	140	2450	140	BQL	140	BQL
8	140	1721	140	BQL	140	BQL	140	BQL
9	140	1526	140	27.8	140	20	140	BQL
10	140	2689	140	5.1	140	N/A	140	N/A

***** BQL: below assay's quantifiable limit (2.1 ng/mL); – Because of disease progression patient did not receive cycle 3 and 4; N/A: data not available

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

These results show that all patients can have good blood levels during cycle 1 and half had detectable blood levels during cycle 2. These results are in agreement with the Roche phase I clinical trial. It is also clear that administration of LMB-100 beyond cycle 2 is unlikely to result in meaningful clinical benefit since there are no detectable blood levels during cycle 3 and 4.

1.2.4 SEL-110

1.2.4.1 Administration of SEL-110 for the Prevention of Anti-Drug Antibodies (ADAs)

As seen above, even with the reduced immunogenicity of LMB-100 compared to SS1P, most patients are only able to complete 1 to 2 cycles of LMB-100 before the level of ADAs prevents achievement of efficacious blood concentrations of LMB-100 in subsequent cycles. Selecta Biosciences is developing a biodegradable nanoparticle drug product containing rapamycin (SEL-

110), which has been shown in preclinical models to induce antigen specific tolerance and has been shown in a Phase 1 clinical study and an ongoing Phase 2 clinical study to prevent the formation of antibodies to a highly immunogenic enzyme when co-administered.

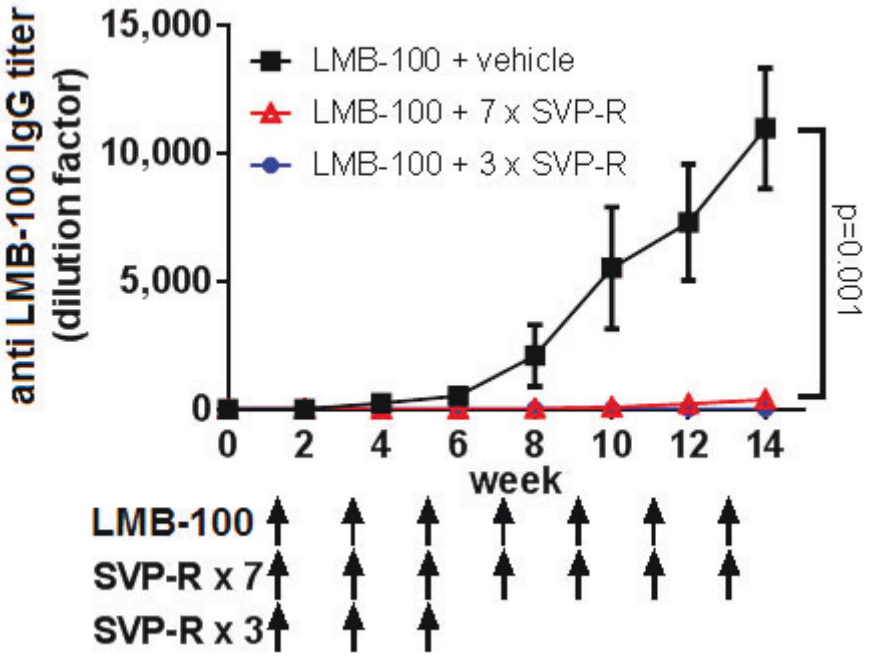
SEL-110 is comprised of rapamycin, a licensed small molecule immunomodulator, encapsulated in a biodegradable PLA-PEG nanoparticle. SEL-110 is designed to inhibit the formation of ADAs when concomitantly administered with a biologic drug during the first few doses of the biologic. Upon i.v. administration, like all nanoparticles, SEL-110 accumulates primarily in the spleen. Once in the spleen the mechanism of action of SEL-110 suggested by evidence to date proposes that the PLA-PEG nanoparticles are avidly taken up by antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, and the subsequent intracellular release of rapamycin, an inhibitor of the mTOR pathway, induces a tolerogenic phenotype in APCs. Dendritic cells are an attractive target for immunotherapies due to their central role in antigen presentation to T-cells and their ability to induce and control regulatory responses to ensure self-tolerance.(25-27) Thomson and colleagues(28, 29) and data from Selecta have demonstrated that treating DCs *in vitro* with rapamycin, induces a tolerogenic DC phenotype capable of inducing Treg differentiation and antigen-specific immune tolerance. Selecta has demonstrated that SEL-110 co-administered with antigen induces tolerogenic DCs and antigen-specific Treg in vivo.(30)

NCI embarked on a preclinical collaboration with Selecta that demonstrated that the application SEL-110 in conjunction with LMB-100 prevented the formation of antibodies to LMB-100 in mouse models. The goal of this clinical trial is to administer SEL-110 with LMB-100 to prevent the formation of antibodies to LMB-100 and allow for effective blood concentrations of LMB-100 to be achieved for multiple cycles.

1.2.4.2 SEL-110 Prevents the Formation of ADAs to LMB-100 in Mouse Model

Female BALB/c mice were injected IV QOW with LMB-100 (2.5 mg/kg) 7 times or a combination of LMB-100 and SEL-110 (2.5 mg/kg) QOW 7 times or the combination 3 times followed by 4 injections of LMB-100 alone (arrows in Figure 4 indicate injections). Plasma was collected and analyzed for anti-LMB-100 antibodies by ELISA. In the groups treated with either 3 injections of SEL-110 in combination with LMB-100 followed by 4 injections of LMB-100 or 7 injections of SEL-110 in combination of LMB-100 the mice did not make appreciable anti-LMB-100 antibodies, whereas the group receiving only LMB-100 had high levels of anti-LMB-100 antibodies. The error bars indicate SEM and the P value was determined by comparing AUC using one way ANOVA (n=8).

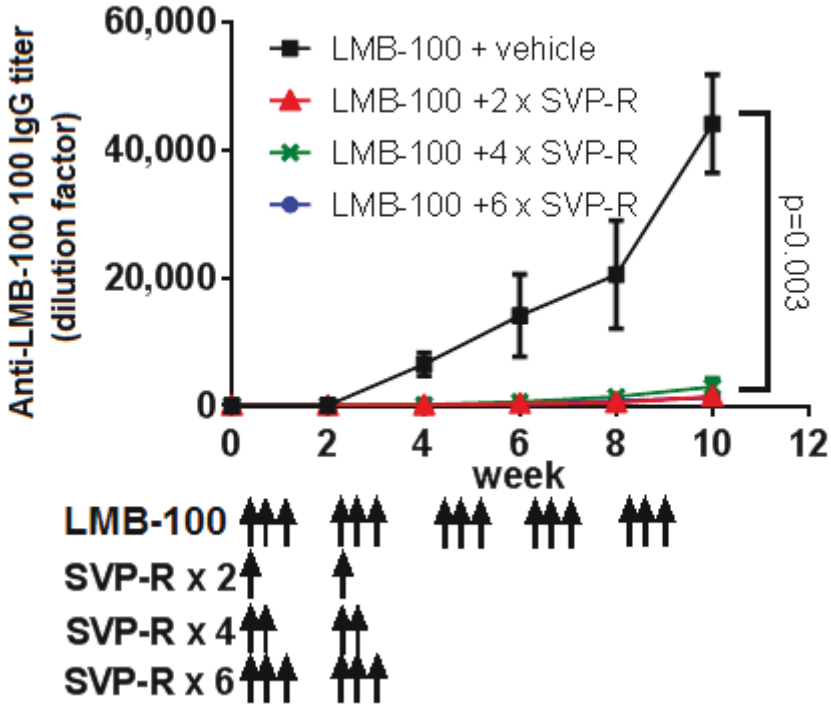
Figure 4. Co-administration of SEL-110 with LMB-100 prevent the formation of anti-LMB-100 antibodies in mice.



1.2.4.3 One Dose Per Cycle is Sufficient to Prevent the Formation of ADAs to LMB-100 in Preclinical Studies

In the clinical setting LMB-100 is given on Days 1, 3 and 5 of each 21-day cycle. In this experiment, we closely mimic the clinical regiment by giving mice LMB-100 IV injections (2.5 mg/kg) on Days 1, 3 and 5 of an every two weeks cycle and giving SEL-110 (2.5 mg/kg) IV with the LMB-100 on Day 1 only, Day 1 and 3 or Day 1,3 and 5 of the first and second cycle. As shown in Figure 5 (error bars are SEM and P value was determined by comparing AUC using one way ANOVA), mice dosed only with LMB-100 made significant anti-LMB-100 antibodies but all groups receiving either 1, 2 or 3 doses of SEL-110 in each of the first two cycles did not produce any anti-LMB-100 antibodies. As such in the proposed clinical design patients will receive LMB-100 and SEL-110 on the first day of the cycle and then just LMB-100 only on days 3 and 5 of each cycle.

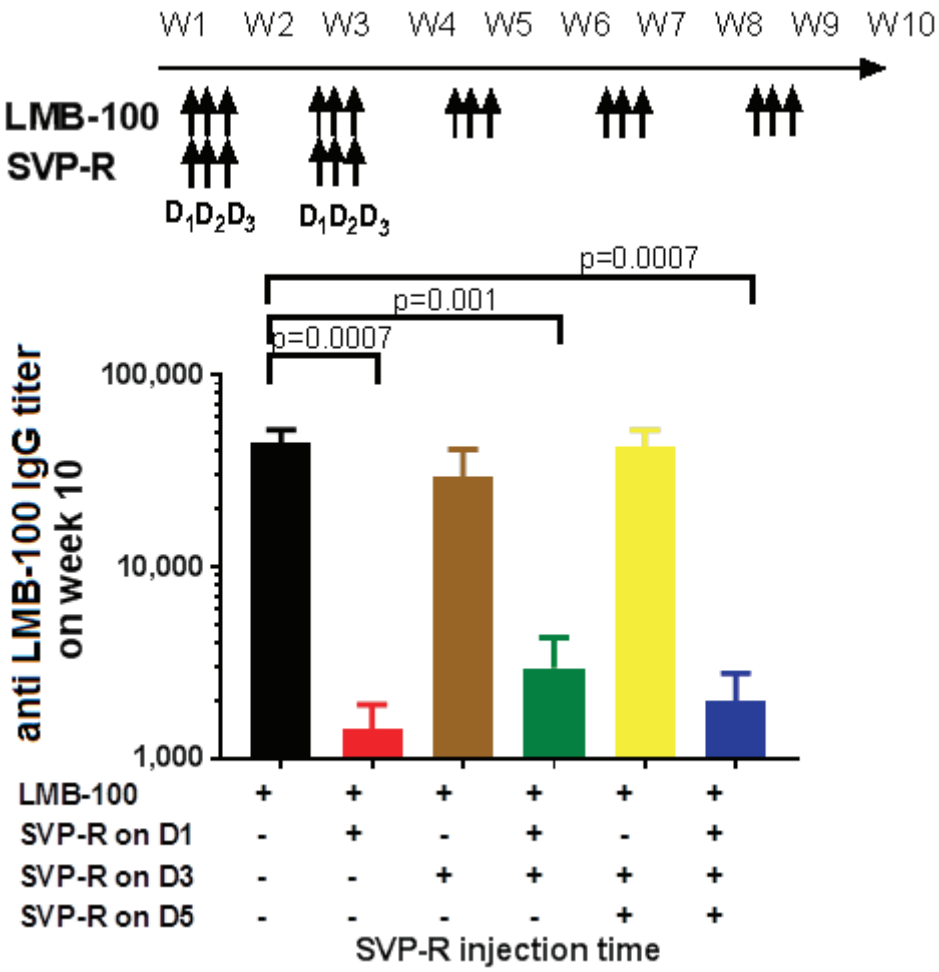
Figure 5. One dose of SEL-110 at the beginning of the first two cycles prevent the formation of anti-LMB-100 antibodies.



1.2.4.4 Administration of SEL-110 on the First Day of the Cycle is Most Effective

Mice were injected IV with LMB-100 (2.5 mg/kg) on Days 1, 3 and 5 of bi weekly cycles for 9 weeks as described in 1.2.7. SEL-110 (2.5 mg/kg) was co-administered IV on the following days of cycles 1 and 2: Day 1, Day 3, Day1 and 3, Day 3 and 5, and Day 1, 3 and 5. The final mean titer at week 10 is shown and P values were determined by one way ANOVA. From Figure 6 it is clear from the data that administration on the first day of the cycle is important and that adding SEL-110 to the second or second and third injection of LMB-100 in a cycle is not advantageous. As such, the proposed clinical protocol administers LMB-100 and SEL-110 on the first day of each cycle and then LMB-100 alone on Day 3 and Day 5 of each cycle.

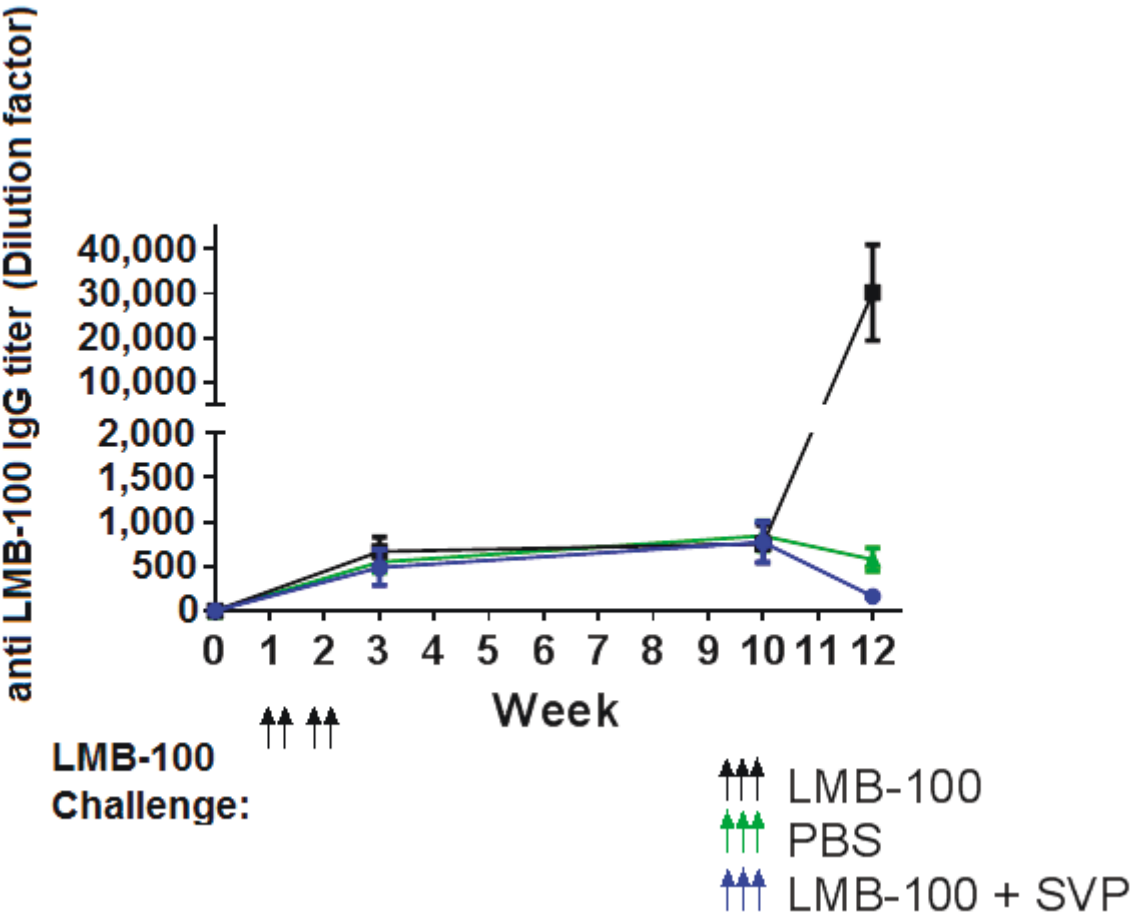
Figure 6. Co-administration of SEL-110 with LMB-100 on the first day of a cycle is key to prevention of anti-LMB-100 antibodies in a mouse model.



1.2.4.5 SEL-110 Prevents Boost of Anti-LMB-100 Antibody Response in Mice with Pre-existing Anti-LMB-100 Antibodies

Female BALB/C mice were injected IV 4 times with LMB-100 (2.5 mg/kg) over two weeks to establish a pre-existing LMB-100 immune response. On week 10 the mice were injected IV with either LMB-100 alone, PBS, or LMB-100 and SEL-110 every other day for a total of 3 injections. Plasma was collected and analyzed for anti-LMB-100 antibodies by ELISA as shown in Figure 7 (error bars are SEM). The mice with pre-existing antibodies had an approximately 30-fold increase in anti-LMB-100 antibody levels while the PBS and LMB-100 plus SEL-110 treated mice saw the anti-LMB-100 antibody levels drop, with the LMB-100 plus SEL-110 group dropping close to baseline. This suggests that SEL-110 could potentially lower pre-existing ADAs to LMB-100 in a patient allowing the patient to receive additional effective cycles of treatment.

Figure 7. SEL-110 prevents the boost of anti-LMB-100 antibody levels in mice with pre-existing anti-LMB-100 antibodies

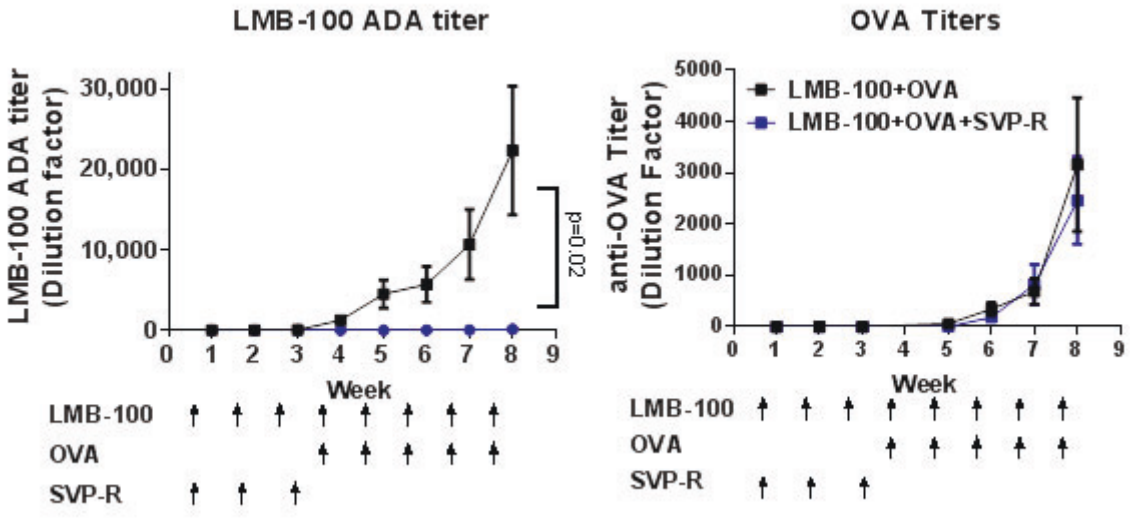


1.2.4.6 Co-administration of LMB-100 and SEL-110 Induces LMB-100 Specific Immune Tolerance in Mice

As opposed to immunosuppression, where all immune responses are suppressed, SEL-110 appears to induce antigen specific tolerance as demonstrated by mice treated with LMB-100 and SEL-110 not making anti-LMB-100 antibodies but making normal ant-OVA antibodies when given the different antigen Ovalbumin (OVA). Mice were injected IV 3 times weekly with LMB-100 (2.5 mg/kg) or with a combination of IV LMB-100 (2.5 mg/kg) with IV SEL-110 (2.5 mg/kg). On weeks 4 to 8 mice were challenged with a weekly IV dose of LMB-100 and a sub-cutaneous dose of Ovalbumin (2.5 mg/kg). Plasma was collected and analyzed for ant-LMB-100 and anti-OVA antibodies by ELISA. Figure 8 shows that the mice treated with LMB-100 alone and then LMB-100 plus OVA made significant levels of antibodies to both LMB-100 and OVA while the mice treated with LMB-100 plus SEL-110 and then with LMB-100 plus OVA did not make antibodies to LMB-100 but made the same, normal immune response to OVA having essentially the same level of anti-OVA antibodies as the group that did not receive SEL-110 (error bars are SEM and

AUC for each curve was calculated and analyzed using Mann-Whitney test, n=5). This demonstrates that the co-administration of SEL-110 plus LMB-100 induced antigen specific tolerance to LMB-100 and did not disrupt normal immune responses to other antigens.

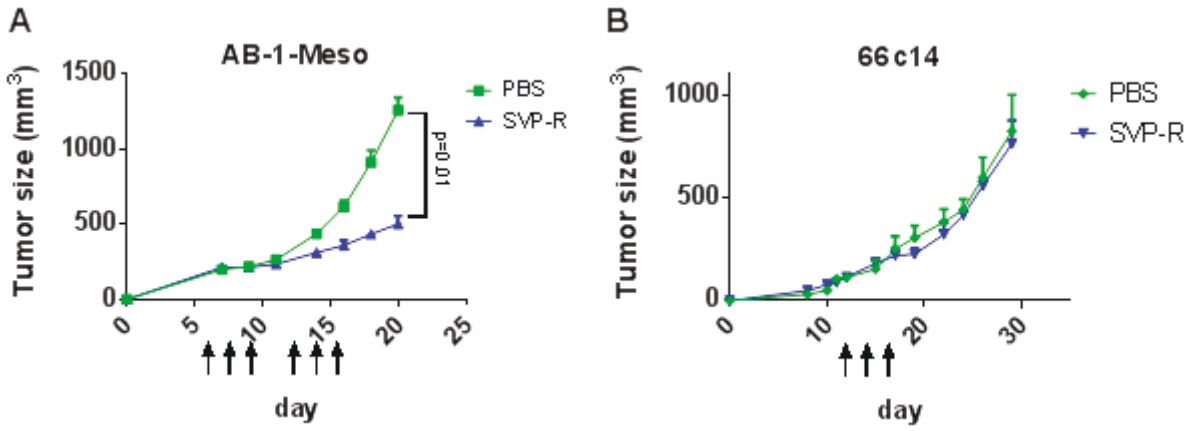
Figure 8. SEL-110 plus LMB-100 induces antigen specific tolerance to LMB-100



1.2.4.7 Administration of SEL-110 Does Not Accelerate Tumor Growth

The administration of SEL-110 does not accelerate tumor growth. Female BALB/c mice were inoculated with AB1 (murine mesothelioma) in the flank. SEL-110 (2.5 mg/kg) or vehicle (saline) were injected IV on days 6, 8, 10, 13, 15 and 17. Tumor size was measured using caliper for 20 days. Figure 9A shows average tumor size (error bars are SEM, n=8 and P value determined by AUC using Mann Whitney test, n=7). The results show that the SEL-110 treated group actually had a slower tumor progression than the PBS treated group. In another mouse tumor model, mice were inoculated with 66c14 (murine breast cancer) in the flank. Tumor size was measured using calipers for 30 days and SEL-110 or vehicle (saline) was IV injected on days 12, 15 and 17. Figure 9B shows average tumor size (error bars are SEM, n=5). In this tumor model the SEL-110 treated mice had tumors that grew at the same rate as the PBS treated mice. In both these tumor models the administration of SEL-110 did not increase the rate of tumor growth.

Figure 9. Administration of SEL-110 does not accelerate tumor growth in two mouse tumor models.



1.2.4.8 SEL-110 Clinical Experience

SEL-110 is being studied as part of Selecta's combination product therapy SEL-212, which combines SEL-110 with SEL-037 (pegsitacase, a peglyated recombinant uricase) for the reduction of serum uric acid levels (sUAs) in patients with severe chronic gout under IND 124184. The study of LMB-100 with SEL-110 will be the first study to evaluate SEL-110 in patients with cancer.

1.2.4.8.1 SEL-212/101 Clinical Trial

SEL-110 was studied alone and in combination with SEL-037 in a single ascending dose Phase 1b study, SEL-212/101. This first study has been completed with final analysis and Clinical Study Report.

For the double-blind SEL-110 portion of the study, a total of 28 subjects with elevated uric acid levels (excluding the potential need for replacement subjects) were dosed in four cohorts in a stepwise, ascending manner. Within each cohort, five subjects received active drug substance and two subjects received placebo (saline). The doses of SEL-110 evaluated were 0.03, 0.1, 0.3, and 0.5 mg/kg.

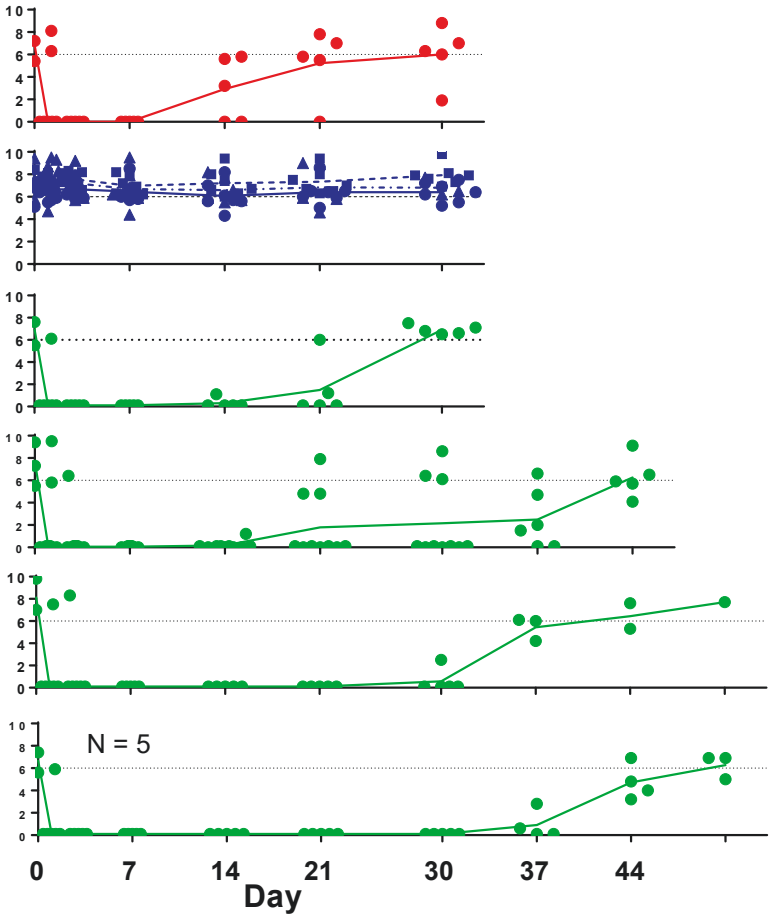
In the open-label SEL-212 portion of the study, six cohorts received a combination of SEL-037 at a dose of 0.4 mg/kg and increasing doses of SEL-110 (0.03, 0.1, 0.15, and 0.3 mg/kg). Adult male and female subjects (N= 36) were enrolled on a dose level (cohort) basis with 5 subjects per cohort, with each cohort administered study drug in an ascending, stepwise manner. Due to study drug-related serious adverse events (SAEs) of stomatitis observed in two subjects in Cohort 7 (0.5 mg/kg SEL-110), the Sponsor did not dose subjects in Cohort 8 at the planned highest dose of SEL-212 (i.e., 0.5 mg/kg SEL-110 + 0.4 mg/kg SEL-037) and determined the maximum tolerated dose (MTD) of SEL-110 to be 0.3 mg/kg in the SEL-212/101 study. Instead, Cohort 12 was added to evaluate subjects at an intermediate dose of SEL-212 (0.15 mg/kg SEL-110 + 0.4 mg/kg SEL-037).

Overall, SEL-110 and SEL-212 was well tolerated by subjects in all cohorts other than Cohort 7. Overall, the majority of TEAEs were mild or moderate in severity and most were assessed as not related to study drug.

Serious AEs were reported in 6 subjects. The events in 4 subjects were assessed as related to study drug. All subjects with SAEs were reported as recovered from the events. One subject (Subject ID 104-0086) who received SEL-212 in Cohort 10 (0.03 mg/kg SEL-110 + 0.4 mg/kg SEL-037) had a non-serious TEAE of an infusion-related reaction that was assessed as moderate in severity and possibly related to study drug; this subject was subsequently withdrawn from study drug. No drug-related SAEs were observed in the two highest dose levels of SEL-212 tested (Cohorts 12: 0.15 mg/kg SEL-110 + 0.4 mg/kg SEL-037, and Cohort 6: 0.3 mg/kg SEL-110 + 0.4 mg/kg SEL-037).

In the open-label portion of the clinical study SEL-212/101, combination product SEL-212 demonstrated the ability to reduce serum uric acid rapidly and to maintain serum uric acid levels below 6 mg/dL for 30 days with the 0.4 mg/kg SEL-037 dose tested. SEL-212 also reduced or prevented the formation of anti-uricase antibodies in a dose-dependent fashion. The uric acid level of all subjects treated with SEL-212 dropped to below 0.1 mg/dL within 24 hours after infusion (Figure 10). SEL-110 appeared to inhibit the formation of anti-uricase antibodies in a dose-dependent manner, which correlated with more sustained control of uric acid levels (Table 8). Evaluation of anti-uricase, anti-PEG, and anti-pegsiticase antibodies showed that anti-uricase antibodies were the most sensitive measure of ADAs in the Phase I study, corresponding with decreased pharmacodynamic activity of SEL-037 as evidenced by increased serum uric acid levels (Table 8).

Figure 10. SEL-212/101 Uric Acid Summary



* Subjects in the 0.1, 0.15 and 0.3 mg/kg groups with <0.1 mg/dL uric acid levels at day 21 were invited on a voluntary basis to return for additional observations after 30 days.

Table 8. Day 30 Serum Uric Acid Levels and Corresponding Anti-Uricase and Anti-PEG Titers

Treatment	Subject ID	Serum UA Level (mg/dL)	Anti-Uricase (Titer)	Anti-PEG (Titer)
0.4 mg/kg SEL-037	108-0010	7	1080	3300.7
	103-0015	6	9720	3013.4
	104-0032	1.9	1080	Neg
	109-0012	6.3	1080	Neg
	104-0036	8.8	9720	Neg
0.03 mg/kg SEL-110 + 0.4 mg/kg SEL-037	104-0012	7.5	120	Neg
	104-0016	6.5	3240	Neg
	107-0016	7.1	1080	Neg
	108-0001	6.6	29160	Neg
	104-0017	6.8	1080	Neg
0.1 mg/kg SEL-110 + 0.4 mg/kg SEL-037	107-0018	<0.1	Neg	Neg
	107-0021	<0.1	Neg	Neg
	104-0027	6.1	29160	Neg
	108-0008	<0.1	120	Neg
	102-0005	<0.1	Neg	Neg
	111-0018	<0.1	120	446.9
	111-0022	8.6	360	419.3
	111-0028	<0.1	Neg	Neg
	111-0029	6.4	9720	1444.9
	106-0004	<0.1	Neg	Neg
0.15 mg/kg SEL-110 + 0.4 mg/kg SEL-037	111-0043	<0.1	Neg	Neg
	111-0045	<0.1	Neg	Neg
	104-0091	<0.1	Neg	Neg
	104-0094	<0.1	Neg	Neg
	111-0049	2.5	9720	Neg
0.3 mg/kg SEL-110 + 0.4 mg/kg SEL-037	107-0027	<0.1	Neg	Neg
	107-0028	<0.1	Neg	Neg
	104-0050	<0.1	Neg	Neg
	104-0060	<0.1	120	Neg
	103-0019	<0.1	Neg	Neg

Pharmacokinetic results are summarized in Table 9 for cohorts of patients who received SEL-110 doses above ≥0.1 mg/kg (i.e., suggested minimum therapeutic dose) and showed dose-dependent responses to SEL-110 with potential influence by co-administration with SEL-037.

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Table 9. Summary of SEL-110 Whole Blood PK Parameters

Parameter	Statistic	Cohort 3 0.1 mg/kg SEL-110 (alone)	Cohort 4 0.1 mg/kg + SEL-110 0.4 mg/kg SEL-037	Cohort 5 0.3 mg/kg SEL-110 (alone)	Cohort 6 0.3mg/kg SEL-110 + 0.4mg/kg SEL-037	Cohort 7 0.5 mg/kg SEL-110 (alone)
C_{max}	N	4	5	5	5	4
(ng/mL)	Mean (SD)	558.5 (181.92)	631 (177.87)	1174 (326.81)	2434 (428.8)	2464.3 (463.91)
	Median	621.5	582.3	1076	2320	2293
	Min, Max	291, 700	495, 942	854, 1640	1942, 3096	2142, 3130
T_{max}	N	4	5	5	5	4
(h)	Median	1.0	1.0	1.0	1.0	1.0
	Min, Max	1.0, 1.5	1.0, 1.1	1.0, 1.0	1.0, 2.0	1.0, 1.0
	N	4	5	5	5	4
$AUC_{0\text{-last}}$		6671.4	10332	18043	37015	37538
(h*ng/mL)	Mean (SD)	(2006.87)	(4912.1)	(7908.5)	(7920)	(4543.7)
	Median	6811.0	8178	19620.0	36848	39222
	Min, Max	4187, 8877	6223, 18548	7056, 28308	25217, 46916	30827, 40881
	N	3	3	4	5	4
$AUC_{0\text{-inf}}$		6596	7818	23241	37950	39813
(h*ng/mL)	Mean (SD)	(1747.1)	(901.59)	(6277.7)	(7590.1)	(5077.8)
	Median	6566	8126	22759	37635	41569
	Min, Max	4865, 8358	6802, 8524	16065, 31381	26629, 47451	32363, 43749
$t_{½z}$	N	3	3	5	5	4
(h)	Mean (SD)	280.2 (18.36)	299.7 (60.73)	214.7 (131.67)	284.4 (54.46)	207.6 (23.68)
	Median	287.7	322.4	290.6	278.5	212.1
	Min, Max	259, 294	231, 346	24.7, 333	235, 375	177, 229
CL	N	3	3	4	5	2

Parameter	Statistic	Cohort 3 0.1 mg/kg SEL-110 (alone)	Cohort 4 0.1 mg/kg + SEL-110 0.4 mg/kg SEL-037	Cohort 5 0.3 mg/kg SEL-110 (alone)	Cohort 6 0.3mg/kg SEL-110 + 0.4mg/kg SEL-037	Cohort 7 0.5 mg/kg SEL-110 (alone)
(L/h)	Mean (SD)	1.415 (0.16299)	1.139 (0.14129)	1.159 (0.22069)	0.9555 (0.36861)	1.217 (0.15249)
	Median	1.372	1.219	1.144	0.9661	1.161
	Min, Max	1.28, 1.6	0.976, 1.22	0.905, 1.44	0.569, 1.46	1.1, 1.44
V_d	N	3	3	4	5	4
(L)	Mean (SD)	574.4 (99.00)	489.8 (105.15)	433.6 (159.46)	380.1 (114.56)	360.9 (18.04)
	Median	569.5	407	454.8	396.5	362.8
	Min, Max	478, 676	453.9, 608	220, 604	204, 495	338, 380

Abbreviations: $AUC_{0\text{-inf}}$ = area under the plasma concentration-time curve from time 0 to infinity; $AUC_{0\text{-last}}$ = area under the plasma concentration-time curve from time 0 to the time t of the last quantifiable concentration; CL = total plasma clearance; C_{max} = maximum observed plasma concentration; max = maximum; min = minimum; NA = not applicable; PK = pharmacokinetics; SD = standard deviation; $t_{½z}$ = apparent terminal elimination half-life; T_{max} = time of maximum observed plasma concentration; V_d = distribution volume in the terminal elimination phase; n = number of actual reliable observations, may be lower for elimination parameters due to exclusion of unreliable PK parameters based on PK acceptance criteria as described in SAP.

1.2.4.8.2 SEL-212/201 Clinical Trial

SEL-110 is also being currently studied as part of SEL-212 combination therapy in an ongoing multi-dose Phase 2 clinical study under IND 124184.

The Phase II study of SEL-212 (Clinical study SEL-212/201) was designed as an open-label, multiple-dose clinical study of the combination drug, SEL-212, which was combined with an open-label, multiple-dose evaluation of pegsiticase alone (SEL-037). Cohorts included patients receiving SEL-037 alone, patients receiving 3 combination doses of SEL-110 plus SEL-037 each 28 days apart followed by two doses of SEL-037 alone each 28 days apart and patients receiving 5 combination doses of SEL-110 plus SEL-037 each 28 days apart. The primary endpoint of the study is safety and tolerability od SEL-212. Additional assessments include the ability of SEL-212 to reduce serum uric acid levels and prevent anti-drug antibodies to uricase, PEG, and pegsiticase as well as PK of rapamycin.

The planned enrollment for the study is now estimated to be approximately 140 subjects among 14 dosing cohorts, each consisting of 6-20 subjects. It is important to note that two dose levels of SEL-037 (0.2 and 0.4 mg/kg) have been evaluated thus far with each ascending dose level of SEL-110 (Table 10) in order to define the minimally effective dose levels of each component of the combination SEL-212 product. Based on the efficacy data acquired to date, future cohorts are now planned with a dose level of SEL-037 of 0.4 mg/kg only. Dose escalation will proceed in a stepwise manner to 0.15 mg/kg of SEL-110. A summary of safety and efficacy data to date from the ongoing study to support this proposed dose escalation regimen is provided as follows: The first subject was treated on 18 October 2016. As of 30 November 2017, 83 patients have been enrolled and treated as summarized in Table 10. At this time Cohorts 1-8 have completed enrollment. Remaining cohorts will continue to enroll until they are closed due to enrollment caps or the stopping rules for individual cohorts.

Table 10. Summary of Enrollment by Treatment Cohort (as of 30 November 2017)

Treatment	Subjects (n)	Treatment	Subjects (n)
Cohort 1 (SEL-037 only) 0.2 mg/kg SEL-037	3	Cohort 2 (SEL-037 only) 0.4 mg/kg SEL-037	3
Cohort 3 (SEL-212) 0.05 mg/kg SEL-110+ 0.2 mg/kg SEL-037	9	Cohort 4 (SEL-212) 0.05 mg/kg SEL-110+ 0.4 mg/kg SEL-037	10
Cohort 5 (SEL-212) 0.08 mg/kg SEL-110+ 0.2 mg/kg SEL-037	6	Cohort 6 (SEL-212) 0.08 mg/kg SEL-110+ 0.4 mg/kg SEL-037	11
Cohort 7 (SEL-212) 0.10 mg/kg SEL-110+ 0.2 mg/kg SEL-037	11	Cohort 8 (SEL-212) 0.10 mg/kg SEL-110+ 0.4 mg/kg SEL-037	10
Cohort 10 (SEL-212) 0.125 mg/kg SEL-110+ 0.4 mg/kg SEL-037	10	Cohort 12 (SEL-212) 0.15 mg/kg SEL-110+ 0.4 mg/kg SEL-037	10
Cohort 11 (SEL-212) 0.15 mg/kg SEL-110 + 0.2 mg/kg SEL-037	0	Cohort 13 (SEL-212) 0.15 mg/kg + 0.2 mg/kg SEL-037	0
Cohort 15 (SEL-212) 0.15 mg/kg SEL-110 (first dose), 0.1 mg/kg SEL-110 (subsequent doses) + 0.2 mg/kg SEL-037	0	Cohort 17 (SEL-212) 0.1 mg/kg SEL-110 + 0.2 mg/kg SEL-037	0
Cohorts 9 and 11 are intentionally omitted from this protocol in order to maintain the cohort numbering convention of associating even numbered cohorts with the 0.4 mg/kg SEL-037 dose.			

Preliminary analyses of safety data are available for the 83 subjects enrolled and treated as of 30 November 2017, with approximately 215 study drug treatments. SAEs characterized as related to study drug have involved anaphylactic reactions or infusion-related reactions in 7 subjects that occurred with temporal proximity to initiation of the second dose of either SEL-037 in Cohort 1 (1 subject, 0.2 mg/kg), SEL-037 in Cohort 2 (1 subject, 0.4 mg/kg), SEL-212 in Cohort 3 (2 subjects at 0.05 mg/kg SEL-110 + 0.2 mg/kg SEL-037), SEL-212 in Cohort 7 (1 subject at 0.1 mg/kg SEL-110 + 0.2 mg/kg SEL-037), and SEL-212 in Cohort 8 (1 subject at 0.1 mg/kg SEL-110 + 0.4 mg/kg SEL-037) and with temporal proximity to initiation of the first dose of SEL-212 in Cohort 6 (1 subject at 0.08 mg/kg SEL-110 + 0.4 mg/kg SEL-037).

All patients were treated with antihistamines, bronchodilators, and/or corticosteroids and all were reported as having recovered from the events. No deaths have been reported. Two withdrawals due to adverse events (other than the listed SAEs) have been reported, both for infusion reactions, one in Cohort 6 and one in Cohort 10. After the observation of the anaphylactic reactions and infusion reaction in Cohorts 1-3, the stopping rules were modified so that additional doses of SEL-212 or SEL-037 would not be administered if sUA levels rose above 6 mg/dL 21 days after the previous dose, and then further restricted to a sUA level threshold of 1 mg/dL at 21 days after the previous dose. The rise in SUA at day 21 is predictive of the presence of ADAs, based on the Phase Ib study of SEL-212. During the period in which the modified stopping rule was being implemented, 5 subjects in Cohorts 1-4 missed their dosing window for the second dose and were removed from the study. Those subjects removed from Cohorts 3 and 4 were replaced, while dosing in Cohorts 1 and 2 was terminated.

To date, 215 single and repeat doses of study drug (SEL-212 or SEL-037) have been administered. Following the modification of the stopping rules to a 1 mg/dL threshold at day 21 post-dose, one anaphylactic event was observed in Cohort 8, which was the result of a protocol violation wherein the subject was administered a second dose of SEL-212 despite having met the stopping criteria. A second anaphylactic event occurred in a patient in Cohort 7 during treatment cycle 2 despite passing the stopping rules. A single infusion reaction event occurred in a Cohort 6 patient who was receiving SEL-110 for the first treatment prior to receiving SEL-037. It is expected that the risk of anaphylaxis will decrease with increasing doses of SEL-110 due to inhibition of ADA formation.

Preliminary analyses of pharmacodynamic data show that SEL-037 administered alone at 0.2 mg/kg (Cohort 1) and 0.4 mg/kg (Cohort 2) reduced sUA (in green) through Day 7, but reduced levels of sUA were not maintained in the physiologic context of rising anti-uricase antibody (in blue) titer (Figure 11). In Cohorts 3, 4, 5, 6, 7,8, 10, and 12, preliminary analyses of pharmacodynamic data as of 30 November 2017 show that SEL-037 administered at 0.2 mg/kg and 0.4 mg/kg in combination with SEL-110 (0.05 mg/kg, 0.08 mg/kg, 0.1 mg/kg, 0.125 mg/kg, and 0.15mg/kg) (i.e., SEL-212) reduced sUA levels and maintained low sUA levels in the physiological context of low anti-uricase antibody titers. A clear relationship between rising sUA and concurrently rising anti-uricase antibody titers was observed in some subjects, and suggests that preventing the formation of ADAs by concomitant administration of SEL-110 at the start of treatment with SEL-037 will induce persistent immune tolerance, such that subsequent treatment with SEL-037 alone does not evoke an immune reaction that may compromise its pharmacodynamic activity. Data for cohort 6 and cohort 8 are shown Figure 12 and Figure 13 respectively.

Thus, on the basis of these data, an initial efficacy signal has been demonstrated with repeated dosing of SEL-212 as evidenced by persistent suppression of serum uric acid levels below 6 mg/dL and anti-uricase specific antibody suppression in conjunction with increasing the dose of SEL-110 up to 0.1 mg/kg. Based on the Phase Ib study, it is anticipated that further increases in SEL-110 would result in more complete inhibition of ADAs, which may potentially reduce the incidence of infusion reactions upon repeat dosing.

Figure 11. Interim sUA and Anti-Uricase Titer: Cohort 1 (0.2 mg/kg SEL-037) and Cohort 2 (0.4 mg/kg SEL-037)

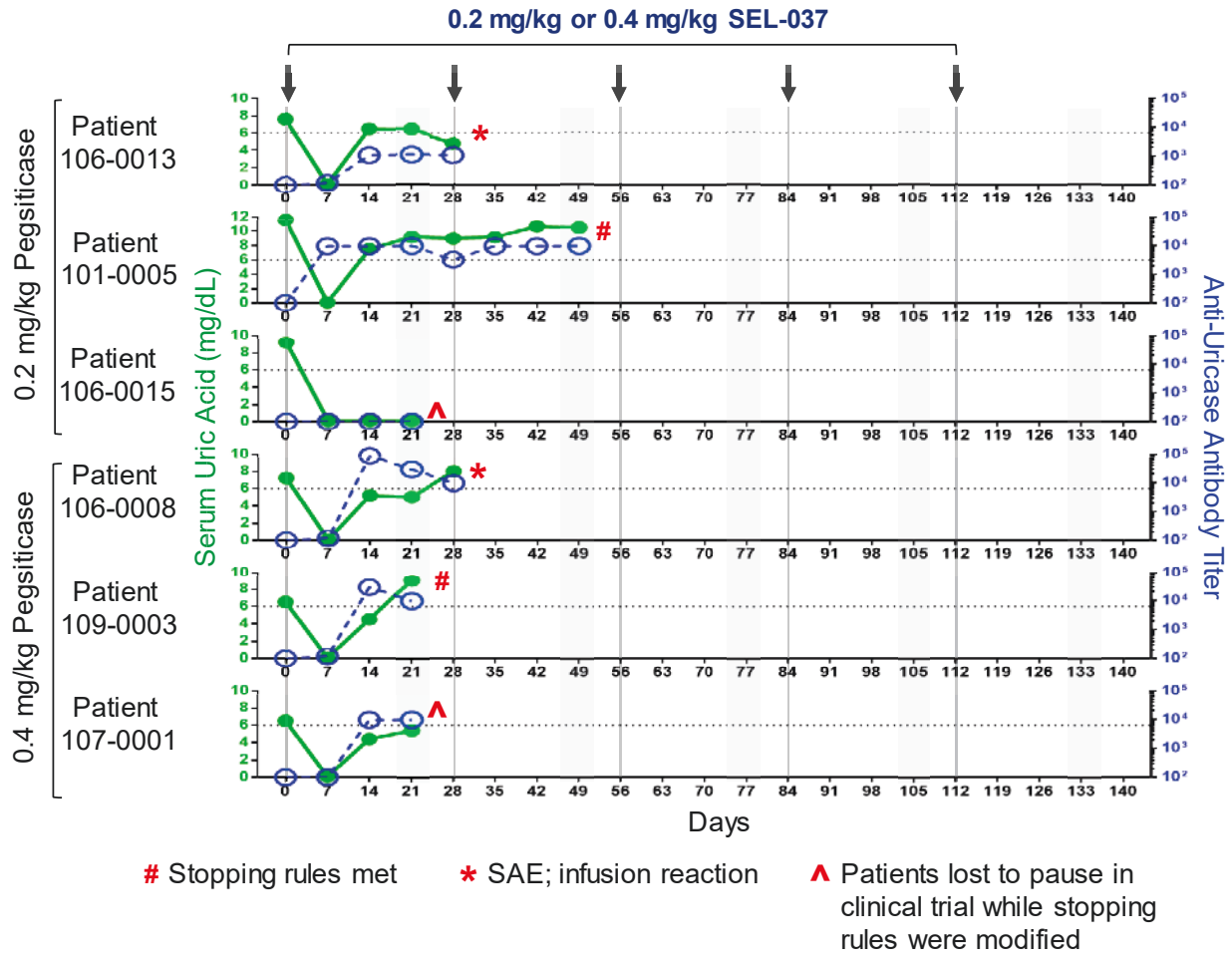
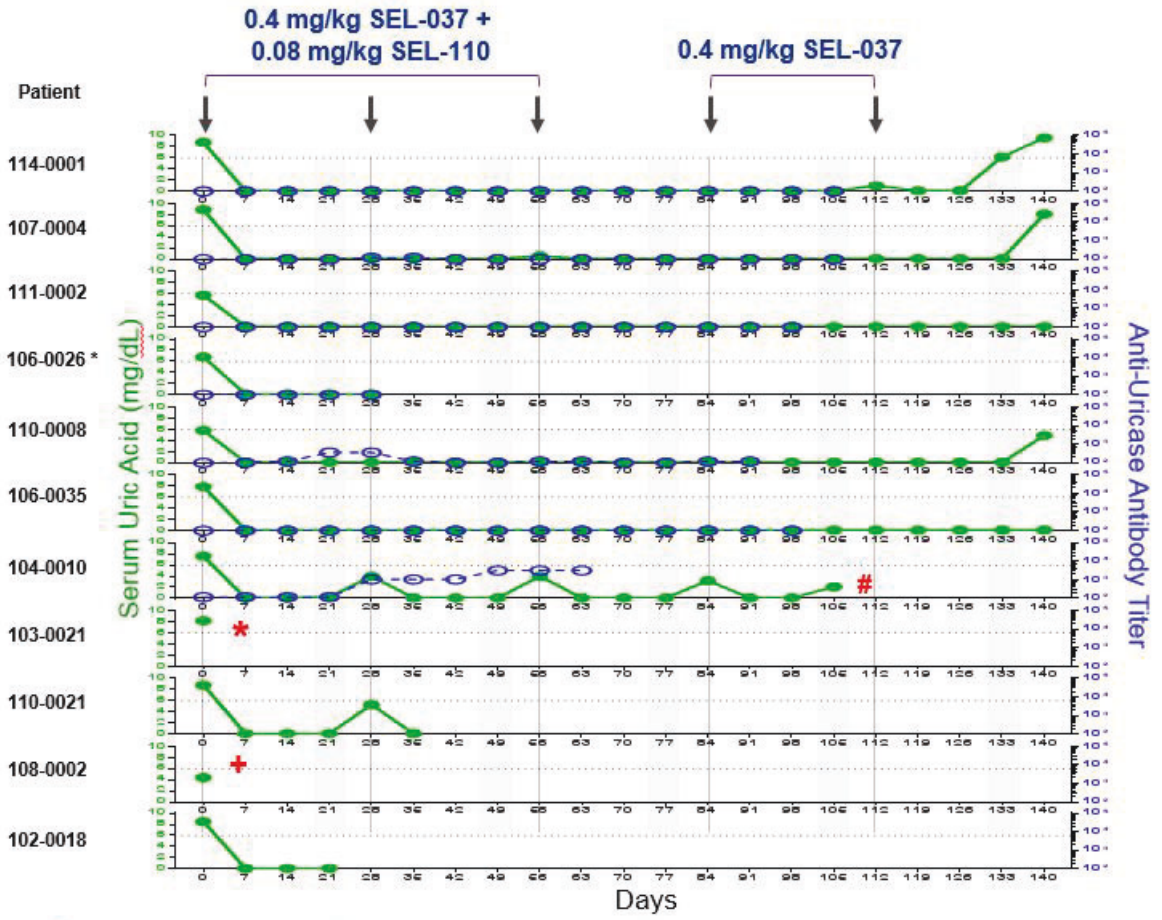


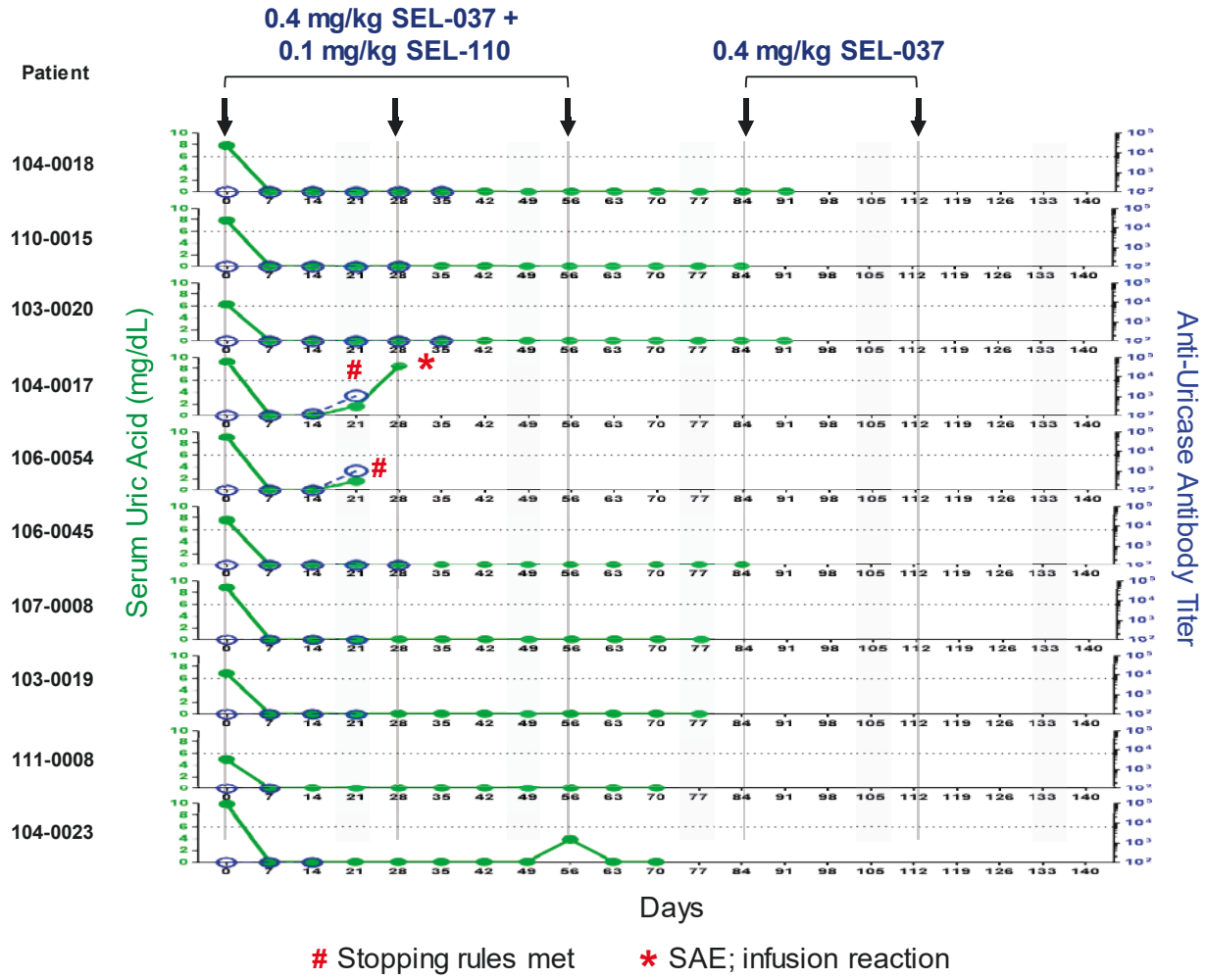
Figure 12. Interim sUA and Anti-Uricase Titer: Cohort 6 (0.08 mg/kg SEL-110 + 0.4 mg/kg SEL-037)



Stopping rules met * SAE; infusion reaction + Discontinuation due to Infusion reaction

* Patient 106-0026 received live attenuated vaccine for shingles (protocol deviation). The patient was withdrawn from the study and was replaced per protocol.

Figure 13. Interim sUA and Anti-Uricase Titer: Cohort 8 (0.1 mg/kg SEL-110 + 0.4 mg/kg SEL-037)



The data from this open label Phase 2 clinical study show that the administration of SEL-110 with the pegylated enzyme SEL-037 prevents the formation of anti-drug antibodies in a majority of patients at the relatively low dose of 0.08 mg/kg. Dose escalation is continuing in this trial and improved efficacy at higher doses is expected.

1.2.5 Rationale for the study

There is clearly an unmet need to identify improved therapies for patients with malignant mesothelioma. LMB-100 is a reduced immunogenicity anti-mesothelin RIT developed in NCI's Laboratory of Molecular Biology in collaboration with Roche. Mesothelin is expressed in over 95% of epithelioid subtype of malignant mesothelioma. Limited dose escalation completed to date indicates safety and provides preliminary data on immunogenicity. The immunogenicity data suggests that despite the reduced immunogenicity of LMB-100 compared to SS1P, significant

numbers of patients can only receive one or two cycles of LMB-100 before the level of ADAs to LMB-100 prevent future doses from being effective. For anti-tumor efficacy it is likely patients will need more than two cycles of LMB-100 with adequate serum concentration of the drug. Although it is hard to predict what is the minimum number of LMB-100 cycle that need to be administered we think that at least four cycles will provide adequate drug exposure for efficacy. There is strong preclinical evidence that the addition of SEL-110 to the LMB-100 can prevent the formation of ADAs and thus allow patients to receive multiple effective dosing cycles of LMB-100. Additionally, clinical data from Selecta Biosciences, with a different antigen but still a very immunogenic enzyme, has demonstrated clinically the ability to prevent ADAs. Together this gives strong support to conduct a Phase 1 clinical trial of the LMB-100 and SEL-110 combination to explore if the combination can allow patients to take multiple cycles of LMB-100 treatment and achieve significant tumor regression. If safe and successful this approach could be used to treat other mesothelin expressing cancers and later expanded to other centers.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

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

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

2.1.1.3 Patients must have measurable disease per RECIST 1.1. See section 6.3.1.1.

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

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

2.1.1.6 Patients for whom no standard curative therapy exists

2.1.1.7 Age greater than or equal to 18 years. Because no dosing or adverse event data are currently available on the use of LMB-100 + SEL-110 in patients <18 years of age, children are excluded from this study

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

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

2.1.1.10 Adequate hematological function: neutrophil count of more than or equal to 1.0 x 10^9 cells/L, platelet count of greater than or equal to 100,000/mcL, hemoglobin more than or equal to 9 g/dL

2.1.1.11 Adequate liver function: Bilirubin less than or equal to 2.5 x the upper limit of normal (ULN) (excluding Gilbert s Syndrome, see below).

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

a. Unconjugated hyperbilirubinemia noted on several occasions

b. No evidence of hemolysis (normal hemoglobin, reticulocyte count, and LDH)

c. Normal liver function tests

d. Absence of other diseases associated with unconjugated hyperbilirubinemia

2.1.1.13 Adequate renal function: creatinine clearance (by Cockcroft Gault formula – see Appendix B) greater than or equal to 50 mL/min.

2.1.1.14 Must have serum albumin > 2.5 g/dL without intravenous supplementation

2.1.1.15 Must have left ventricular ejection fraction > 50%

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

2.1.1.17 The effects of LMB-100 in combination with SEL-110 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry until 3 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

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

2.1.2 Exclusion Criteria

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

2.1.2.2 Evidence of significant, uncontrolled concomitant diseases which could affect compliance with the protocol or interpretation of results, including significant pulmonary disease other than primary cancer, uncontrolled diabetes mellitus, and/or significant cardiovascular disease (such as New York Heart Association Class III or IV cardiac disease, myocardial infarction within the last 6 months, uncontrolled arrhythmias,

unstable angina, non-compensative congestive heart failure, or clinically significant pericardial effusion)

2.1.2.3 Active or uncontrolled infections.

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

2.1.2.5 Patients with prior pneumonectomy

2.1.2.6 Prior therapy with LMB-100

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

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

2.1.2.9 Dementia or altered mental status that would prohibit informed consent

2.1.2.10 Live attenuated vaccinations 14 days prior to treatment

2.1.2.11 Pregnant women are excluded from this study because it is unknown whether LMB-100 + SEL 100 has 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+SEL-110, breastfeeding should be discontinued if the mother is treated with LMB-100+SEL-110. These potential risks may also apply to other agents used in this study.

2.1.2.12 Known hypersensitivity to any of the components of LMB-100 and/or SEL-110

2.1.2.13 Presence of immunosuppressive conditions, including administration of any medications or treatments that may adversely affect the immune system such as allergy injections, immune globulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids (oral or injectable) during 3 months prior to enrollment. Inhaled and topical corticosteroids allowed.

2.1.2.14 Known allergy to PEGylated products.

2.1.2.15 History of anaphylactic reaction to a recombinant protein or hypersensitivity to PEG.

2.1.2.16 Taken an investigational drug within 4 weeks prior to study drug administration or plans to take an investigational agent during the study.

2.1.2.17 Taken a strong inhibitor or inducer of CYP3A4 within 14 days prior to enrollment (see Flockhart Table or similarly updated source for a list of such agents)

2.1.2.18 Taken drugs known to interact with Rapamune such as cyclosporine, diltiazem, erythromycin, ketoconazole (and other antifungals), nicardipine (and other calcium channel blockers), rifampin, verapamil within 14 days prior to enrollment

2.1.2.19 Uncontrolled hypertension (above 150/95 mm Hg).

2.1.2.20 History of end-stage renal disease requiring dialysis.

2.1.2.21 Serum phosphorus less than 2.0 mg/dL

2.1.2.22 Organ transplant recipient

2.1.3 Recruitment Strategies

Information about the study will be posted on sites such as clinicaltrials.gov and the CCR recruitment website. 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

Screening assessments will be performed within 28 days prior to study enrollment unless otherwise indicated. Assessments may be performed on an NIH screening protocol.

- Archival tumor sample for NCI LP confirmation of diagnosis (at any time prior to enrollment). A block of primary tissue (or 5-10 unstained sections on charged slides) from the time of diagnosis will be required from each patient. Tissue blocks from a known recurrence will be accepted if original tumor samples are unavailable. Referring institutions will send the tumor block or 5-10 unstained sections on charged slides to CCR/NCI for correlative studies and confirmation of diagnosis. A fresh biopsy or tumor effusion sample may be collected if archival tumor tissue is not available.
- History and physical exam
- Vital signs including pulse oximetry
- ECOG performance status
- Urine or serum hCG in women of childbearing potential
- ECG
- Echocardiogram
- CT scan of chest, abdomen and/or pelvis and areas of known or suspected disease involvement; MRI may also be performed when appropriate
- FDG-PET scan
- CBC with differential, Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Lipid Panel (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol), creatine kinase, C-reactive protein, Coagulation (PT, PTT, fibrin degradation products)
- Urinalysis
- Viral markers – HBSAg, anti-HCV, anti-HIV

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site

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

2.4 TREATMENT ASSIGNMENT PROCEDURES

Cohorts

Number	Name	Description
1	Dose escalation	The first 12 eligible and evaluable patients will be treated with a fixed dose of LMB-100 and escalating doses of SEL-110
2	Dose Expansion	Up to 6 additional eligible and evaluable patients will be treated with highest safe dose observed in Arm 1/Cohort 1

Arms

Number	Name	Description
1	Dose Escalation	LMB-100 + SEL-110 (escalating doses)
2	Dose Expansion	LMB-100 + SEL-110 (at dose determined in dose escalation portion of the study)

Stratifications, Randomization and Arm Assignment

No stratification of randomization will occur on the study.

Patients in Cohort 1 will be directly assigned to Arm 1.

Patients in Cohort 2 will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase I, inpatient/outpatient, single center, dose escalation study of LMB-100 in combination with SEL-110. Two different doses of SEL-110 will be evaluated in combination with a fixed dose of LMB-100. Up to 4 cycles (1 cycle = 21 days) of combination therapy may be administered. SEL-110 will be administered intravenously on day 1 of each 21-day cycle. LMB-100 will be administered intravenously on days 1, 3 and 5 of each 21-day cycle.

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

3.1.1 Dose Limiting Toxicity

For the purpose of the study, a DLT will be defined as any of the following events occurring within 21 days after the first dose of LMB-100 or SEL-110, the DLT evaluation period. Toxicities determined to be unequivocally related to disease progression or intercurrent illness will not be regarded as DLTs.

Hematological toxicities:

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

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

- Alopecia (any grade)
- Grade 3 nausea and vomiting lasting >48 hours despite appropriate treatment
- Grade 3 diarrhea lasting for ≤ 2 days with no fever or dehydration
- Infusion-related reactions **up to and including Grade 3**. They are not considered to be DLTs since, based on experience with monoclonal antibodies, IRRs are idiosyncratic and not dose-related events. Precautions will be taken if IRRs Grade ≥ 2 occur (see Section 3.3).
- Laboratory values of ≥ grade 3 that are judged not clinically significant by the investigator.
- **Isolated** Grade 3 fever **(without signs and/or symptoms of an infection)** occurring within 48 hours after LMB-100 infusion and resolving within 48 hours to ≤ Grade 2 and fully resolved within 1 week

Other toxicities:

- Any other drug related toxicity considered significant enough to be qualified as a DLT in the opinion of the principal investigator.

- Inability to start cycle 2 within 3 weeks after completing cycle 1 due to drug-related adverse events.

If a patient experiences a DLT, the patient will be removed from study therapy.

3.1.2 Dose Escalation

Dose escalation will proceed in cohorts of 3–6 patients. The MTD is the dose level at which no more than 1 of up to 6 patients experience DLT during the first cycle of treatment, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level.

The dose escalation cohorts are as follows:

Dose Level	**LMB-100 Dose (mcg/kg)**	**SEL-110 Dose (mg/kg)**
- 1	100	0.15
-1A	100	0.3
1	140	0.15
2	140	0.3
Dose level -1A to be explored if there is acceptable toxicity (per standard 3 plus 3 design) at dose level -1		

Dose escalation will follow the rules outlined in the Table below.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level with no letter designation if only 3 patients were treated previously at that dose OR up to six (6) additional patients will be entered at the next lowest dose level if next lowest dose level with no letter designation previously unexplored.
1 out of 3	Per PI discretion, follow instructions for ≥ 2 DLT OR Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level.

Number of Patients with DLT **at a Given Dose Level**	**Escalation Decision Rule**
	• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. UP to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

3.2 DRUG ADMINISTRATION

3.2.1 SEL-110

SEL-110 will be administered intravenously on day 1 of each cycle for up to 4 cycles.

The thawed SEL-110 will be withdrawn from the vial and dosed via IV infusion with an in-line filter set-up along with a syringe infusion pump at a single steady rate to deliver the dose over a period of 55 minutes starting concurrently with a 60-minute infusion of 125 mL of normal saline. The maximum rate of infusion of SEL-110 for the first 30 minutes should not exceed 5.5 mL/hr. If the calculation of the infusion rate to deliver the entire dose is greater than 5.5 mL/hr, then the infusion rate should be set for 5.5 mL/hr for the first 30 minutes, then the infusion rate should be increased to deliver the rest of the dose over the remaining 25 minutes.

Follow instructions below:

1. Convert weight of subject to kg, as needed (weight in pounds divided by 2.20462).
2. Calculate the volume (mL) of SEL-110 required for a subject.

   Volume (mL) SEL-110 required = (dose in mg/kg) x (subject weight in kg) / (concentration of SEL-110 per ml in vial i.e., 2 mg/mL)

3. Calculate the number of vials of SEL-110 required for a subject. Round the calculation up to the next whole number.

   Number of SEL-110 vials required =[Volume (mL) SEL-110 required + priming volume (mL)]/ (5 mL/vial)

4. Thaw the required number of vials at room temperature for approximately 2 hours. Once the vials are thawed, invert slowly (don't shake) each vial twenty times to ensure product is uniform. Do not use any vials with visible clumping. Thawed SEL-110 should be administered as soon as possible but thawed SEL-110 is stable at room temperature and normal light conditions for 24 hours.

5. Load a syringe with the appropriate size for the dose. In order to prime the line between the syringe and the injection site valve, an additional priming volume of SEL-110 (approximately 0.5mL) equal to the volume of the connecting line used and the volume of the 5 micrometer membrane filter (Pall Medical, PN HP1050 or equivalent) should be added to the syringe. When loading syringes, do not bubble air through the product to avoid the formation of foam.

6. Start an infusion of 125 mL of normal saline solution over 60 ± 2 minutes using an IV set with an injection site valve.

7. Using the injection site valve, immediately after starting the normal saline IV, slowly inject the SEL-110 using a syringe infusion pump at a single steady rate to deliver the dose over a period of 55 ± 2 minutes, if this rate is greater than 5.5 mL/hr then set the infusion rate to 5.5 mL/hr for the first 30 minutes then increase the infusion rate to deliver the rest of the dose over the remaining 25 minutes. A 5 µm syringe filter (5 µm syringe filter Supor (PES) membrane, Pall Medical P/N HP1050 or equivalent) for single use should be included in the SEL-110 line downstream of the syringe and proximal to the injection site valve. At the end of the 55 minutes, continue the saline infusion for the additional 5 minutes after the SEL-110 infusion stops to flush the main line. Do not flush the line between the syringe and the injection site valve

    - The 125 mL of normal saline should be delivered from IV bags via the main line while SEL-110 is delivered in parallel to the normal saline via a syringe infusion pump connected to the injection site valve.

Example for subject weighing 200 pounds at 0.3 mg/kg SEL-110

Step 1: If not already done convert weight to kg:	*= 200 pounds / 2.20462 = 90.7 kg*
Step 2: Calculate volume of SEL-110 needed:	*= (0.3 mg/kg) x (90.7 kg) / (2 mg/mL)* *= 27.2/2 = 13.6 mL SEL-110*
Step 3: Calculate number of vials needed:	*= 13.6 mL SEL-110 required / (5 mL/vial)* *= 2.7 vials. Round up to 3 vials.*
Step 4 and 5: Thaw 3 vial and load syringe with 13.6 mL SEL-110 plus the priming volume (assuming prime volume = 0.5mL, giving a total syringe fill volume of 14.1 mL of SEL-110). Thaw an additional vial if necessary to supply the priming volume.	
Step 6: Start infusion of 125 mL of normal saline solution over 60 ± 2 minutes.	
Step 7: Immediately after the start of the normal saline infusion, slowly inject 13.6 mL of SEL-110 using a 5 µm syringe filter proximal to the injection site valve. As the infusion rate to deliver 13.6 mL of SEL-110 over 55 minutes is 14.8 mL/hr (> 5.5 mL/hr), set the infusion rate to 5.5 mL/hr for the first 30 minutes. To deliver the remaining 10.85 mL (13.6 – 5.5/2) of SEL-110 over the last 25 minutes set the infusion rate to 26 mL/hr. At the end of the 55 minutes, continue the saline infusion for the additional 5 minutes after the SEL-110 infusion stops to flush the main line.	

Only if medically warranted in response to an AE will the Investigator modify the infusion parameters (decrease the rate of infusion, interrupt the infusion, or reduce the dose volume

infused). The Investigator will notify the study team of any changes in infusion parameters. Please refer to Table 13.

3.2.2 LMB-100

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

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

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

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

3.2.2.1 General Instructions

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

3.2.2.2 Specific Instructions

LMB-100 is diluted with 0.9% NaCl (1:10) **in-line** immediately prior to administration (see Figure 14 below).

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

In order to allow **in-line dilution (1:10) immediately prior** to administration of drug product, a side flow with 0.9% NaCl must be applied (as illustrated in Figure 14). An IV infusion pump and

syringe driver should be used to control the infusion rate of isotonic 0.9% NaCl solution and LMB-100 respectively.

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

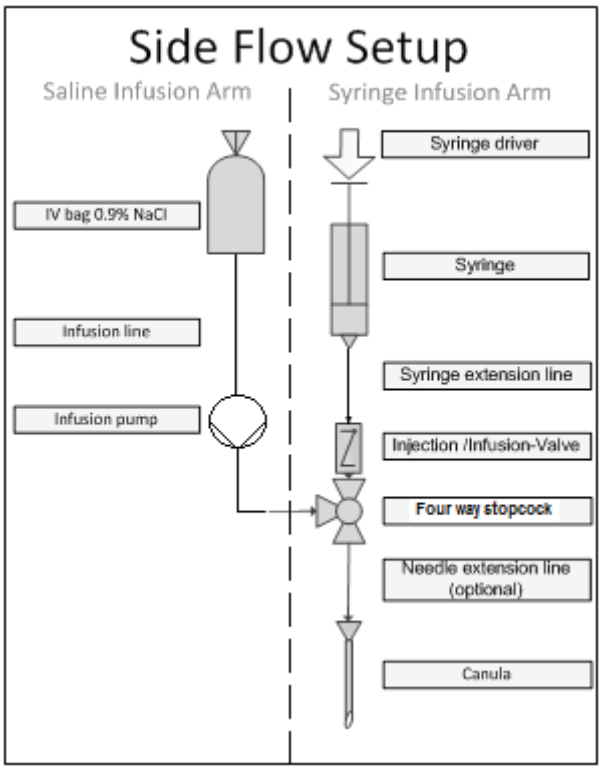


Figure 14. Schematic view of the administration set-up.

The LMB-100 drug product should be filtered during withdrawal from the vial using a BD filter needle.

1. The syringes for administration must be prepared under appropriate aseptic conditions as LMB-100 drug product does not contain antimicrobial preservatives. All preparation should be conducted under laminar flow hood with safety glasses, lab coat and arm protection and a work procedure to minimize particle contamination.
2. As a measure of precaution, a BD 18G (USA: 19G) blunt filter needle Versapore will be used, for the withdrawal of the undiluted drug product (LMB-100) from the vial into the syringe.
3. Withdraw up to 2 ml excess volume of LMB-100 to the intended dosing volume into the syringe for priming and purging the syringe extension lines before administration (see step 7).
4. The prepared syringe should be stored and transported with a closing cone/stopper.

5. Since LMB-100 drug product does not contain antimicrobial preservatives and to comply with the chemical and physical in-use stability, the prepared syringes for infusion should be used immediately. If not used immediately, total in-use storage times of prepared syringes should not exceed 24 hours to limit microbial growth in case of potential accidental contamination. Storage conditions should generally be at 2°C to 8°C, but syringes may be held at room temperature for up to a maximum of 4 hours. The temperature during syringe preparation, storage and drug administration must not exceed 25 °C.

6. Establish the saline flow first by flushing the lines including the 4-way stopcock and extension lines with saline.

7. Put the filled syringe in the pre-programmed syringe driver, remove the cap and attach the infusion set. Follow the syringe driver manual/local instructions regarding how to set up and prime/purge the system before the line is attached to the closed 4-way stopcock before starting the administration.

8. The end of infusion is defined as the time point at which the syringe driver finishes administering the total volume of LMB-100 to be infused.

9. At the end of the first infusion, the IV line should remain in place for 2 hours. If no infusion related symptoms occur during this time, the infusion line may be removed. For subsequent infusions and if no IRR has been reported, the IV line should remain in place for at least 30 minutes from the end of the infusion. If no adverse events occur during the 30 minutes, the infusion line may be removed. If feasible, the line for drawing blood for PK samples (opposite extremity to the one with the infusion line) will remain in place until the 24-hour sample is taken.

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

LMB-100 drug product (DP) should be administered diluted using a side flow set-up at 1:10 (0.1 mg/mL DP). In order to not compromise drug product physico-chemical stability, the dilution with 0.9% NaCl should be done **in line**, immediately prior to administration of the DP. The infusion duration should be 30 minutes (± 5 minutes); **however the duration can be increased at the discretion of the investigator** based on the total dose and volume to be administered and the patient's physical condition. Syringe preparation and infusion duration should not exceed a maximum of 4 hours. In case of any adverse events related to the infusion, please refer to the specific recommendation described in section 3.3.1.

3.2.3 Co-administration of SEL-110 and LMB-100

On days when both LMB-100 and SEL-110 are scheduled to be administered (Day 1 of each cycle), SEL-110 will be administered first. LMB-100 infusion will be initiated within 15 minutes after completion of the SEL-110 administration.

3.2.4 Premedications for Patients Receiving LMB-100

Due to the prevalence of infusion related reactions (IRRs) seen in the previous study of LMB-100, all patients will be premedicated 30-60 minutes (+ 30 minutes) prior to each LMB-100 administration or, on days when both SEL-110 and LMB-100 are administered, prior to each SEL-110 infusion with the following medications:

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

(See section 3.3.1 for complete instructions on response to IRRs)

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

Table 11. Premedication for LMB-100

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

[a] or alternative antihistamine at an adequate dose.

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

3.3 DOSE MODIFICATIONS

3.3.1 LMB-100

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

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

Event	Action to Be Taken
IRR/hypersensitivity reaction	If an IRR/hypersensitivity develops, the infusion of LMB-100 should be temporarily slowed down or interrupted. The patient should be monitored until complete resolution of the symptoms and treated as clinically indicated. Treatment or concomitant medication may include acetaminophen, antihistamine, IV saline, oxygen, bronchodilators, corticosteroids, and vasopressors depending on the symptoms. If the infusion is interrupted: o In the event of IRR CTCAE Grade 1, 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
Vascular leak syndrome	In the event of Grade ≥2 CTCAE vascular 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 each 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	Periodic monitoring of renal function and serum electrolytes IV fluids 0.9 NaCl may be administered after LMB-100 infusion if clinically indicated because of increasing serum creatinine or decreased urine output.
IRR = infusion related reaction; IV = intravenous; CTCAE = Common Terminology Criteria for Adverse Events	

3.3.2 SEL-110

Table 13 below provides a guideline on how to manage certain toxicities which are expected with SEL-110 based on preclinical studies and previous clinical experience with molecules in the same class

Table 13. Guidelines for Managing Known SEL-110 Adverse Events

Event	Action to Be Taken
Leukopenia	○ Leukopenia has been reported in patients taking oral rapamycin. Whether the cause of the leukopenia is a direct relation to the rapamycin exposure, precedes or is due to the development of an opportunistic infection is unknown. Resolution of the leukopenia occurred in cases without subsequent infection with the stoppage of Sirolimus (Rapamycin) exposure. ○ The risk of leukopenia and infection will be addressed in as follows: ▪ The study excluded subjects with any evidence of infection or developing infection by evaluating their vital signs, WBC count and differential. ▪ All subjects were instructed to avoid anyone who has an active infection either bacterial or viral. Subjects also had a WBC performed on day 3, 7, 14, 21 and 30 post exposure. ○ Observation. WBC counts have returned to baseline when rapamycin does have been separated by at least 21 days.

Event	Action to Be Taken
	A complete blood count should be performed in patients where leukopenia is observed. They should also avoid other patients or family members in whom an active infection exists. If the leukopenia resolves, patients have been received additional doses of rapamycin. o As of 30 November 2017, no SAEs or study drug discontinuations involving leukopenia have been reported.
Metabolic disruptions (hyperglycemia, insulin resistance, hypertriglyceridemia and hypercholesterolemia)	o Metabolic disruptions (hyperglycemia, insulin resistance, hypertriglyceridemia and hypercholesterolemia) have been reported in post-transplant patients who have been exposed to rapamycin.(31, 32) The occurrence of post-transplant diabetes mellitus (PTDM) has been recognized for many years as a consequence of solid organ transplant immunosuppression.(33) Dyslipidemia is a well-recognized side effect of rapamycin therapy.(34) o The exact mechanisms of rapamycin induced hyperglycemia, insulin resistance and its lipid effects are unclear at this time. It has been shown that rapamycin can diminish β-cell proliferation in hyperglycemic states thereby inhibiting the natural cellular response of neogenesis, proliferation, hypertrophy and a reduction in apoptosis.(35) Additionally, rapamycin has been shown to inhibit phosphorylation of IRS-1 and IRS-2 in human adipocytes mimicking changes seen in type 2 diabetes and in human peripheral blood monocytes associated with insulin resistance.(36) Rapamycin has also been shown to increase basal lipolysis and reduce lipid storage.(36) o Patients who experience metabolic disturbances (increased glucose, triglyceride and LDL cholesterol levels) should be treated at the discretion of the investigator according to their clinical judgement. Preliminary analyses of final safety data in the clinically complete Phase I study indicate no TEAEs involving metabolic disorders or lipid profiles in subjects who received SEL-212 (combination of SEL-110 and SEL-037).

Event	Action to Be Taken
	○ As of 30 November 2017, no SAEs or study drug discontinuations involving metabolic disorders or changes in lipid levels have been reported.
Hypophosphatemia	○ The mechanism of hypophosphatemia is not known, but it has been seen in patients that have received mTOR inhibitors such as rapamycin.(37) ○ Preliminary analyses of final safety data in SEL-212/101 Phase I study indicate no TEAEs involving hypophosphatemia in subjects who received SEL-212 (combination of SEL- 110 and SEL-037). ○ As of 30 November2017, no SAEs or study drug discontinuations involving hypophosphatemia have been reported
Stomatitis	○ The mechanism of stomatitis is not known, but has been reported in patients that have received mTOR inhibitors such as rapamycin as early as 7 days after treatment and responds to locally applied Clobetasol cream.(37) ○ Analyses of final safety data in the Phase I SEL-212/101 study indicate that SAEs of stomatitis in 2 subjects who received SEL-110 at the highest dose (0.5 mg/kg) were characterized as related to study drug. Both subjects recovered from the events. As a result of these two SAEs, the Sponsor did not dose patients at the planned highest dose of SEL-212 (0.5 mg/kg SEL-110 + 0.4 mg/kg SEL-037). ○ Patients who experience early signs of stomatitis should treat the local lesion with Clobetasol gel or cream directly to the lesion. Initially the appearance is thought to be that of an aphthous ulcer. Early application of the steroid treatment usually results in resolution. Interestingly these lesions don't usually recur after resolution and continued rapamycin therapy. ○ As of 30 November2017, no SAEs or study drug discontinuations involving metabolic disorders or changes in lipid levels have been reported.
Immunologic Responses	○ The risk of infusion reactions are addressed as follows: ▪ Subjects with known allergy to PEGylated products and history of anaphylactic reactions to recombinant

Event	Action to Be Taken
	protein or hypersensitivity to PEG are excluded from study. ▪ Measurements of anti-PEG antibodies will be taken as a baseline upon pre-dose and at end of study. ▪ Each subject will be observed for infusion reactions after dosing of study drug in the clinic for 9 hours. Subjects will be instructed on the signs or symptoms of infusion reaction and told to notify the PI immediately if they feel they are experiencing one. ▪ SEL-110 will be administered via syringe pump to control the infusion rate of the small volume of material. ▪ As of 30 November 2017, SAEs characterized as related to study drug have involved anaphylactic reactions or infusion related reactions in 7 subjects that occurred with temporal proximity to initiation of the second dose of either SEL-037 or SEL-212 (1 subject at 0.1 mg/kg SEL-110 + 0.2 mg/kg SEL-037), and at 0.1 mg/kg SEL-110 + 0.4 mg/kg SEL-037) and with temporal proximity to initiation of the first dose of SEL-212 in Cohort 6 (1 subject at 0.08 mg/kg SEL-110 + 0.4 mg/kg SEL-037). All patients were treated with antihistamines, bronchodilators, and/or corticosteroids and all patients were reported as having recovered from the events. Two withdrawals due to non-severe, non-serious adverse events of infusion reactions (other than SAEs) have been reported, one each in Cohort 6 (0.08 mg/kg SEL-110 + 0.4 mg/kg SEL-037) and Cohort 10 (0.125 mg/kg SEL-110 + 0.4 mg/kg SEL-037). Six additional non-severe, non-serious adverse events of infusion reactions were reported by 5 subjects in Cohort 3 (1 subject at 0.05 mg/kg SEL-110 + 0.2 mg/kg SEL-037), Cohort 5 (1 subject at 0.08 mg/kg SEL-110 + 0.2 mg/kg SEL-037), Cohort 6 (1 subject at 0.08 mg/kg SEL-110 + 0.4 mg/kg SEL-037), and Cohort 7 (2 subjects at 0.1 mg/kg SEL-110 + 0.2 mg/kg SEL-037) which did not result in withdrawal of study drug. Only two subjects required treatment with antihistamines and corticosteroids, and all subjects were reported as having recovered from the events.

Event	Action to Be Taken
Reproductive System Disruptions	o Oligo/azoospermia has been seen in some male renal transplant patients taking an oral form of SEL-110 (rapamycin) administered chronically on a daily or three times weekly basis for the prevention of transplant rejection when treated for a period greater than 6 months. The mechanism for this effect is not known. In those patients where oligo/azoospermia has been seen partial to full reversibility of sperm production following cessation of treatment with the oral form of rapamycin has been demonstrated. o Nonclinical data from three repeat dosing of 6 mg/kg SEL-110 in male rats showed return of spermatogenesis and normal levels of sperm in many rats, suggesting recovery of the original testicular effects, while in some rats the presence of atrophic tubules at the end of the recovery period may have represented partial recovery or may have been related to normal senescence. o Male patients should be informed that their fertility may be affected temporarily or permanently after being given SEL-110. o In patients prescribed chronic daily Rapamune® (same active ingredient as SEL-110, rapamycin) cessation of rapamycin has resulted in some of the cases experiencing a partial or complete resolution to their oligospermia. No information on recovery of patients taking SEL-110 is currently available.
Fetal Development	o In preclinical animal studies performed with an oral form of the drug to be administered in this study, SEL-110 (a component of SEL-212) showed adverse effects when given to pregnant rats. These effects included fetal death, reduced fetal weights, and delays in the formation of bones. o It is not known whether these effects would also occur humans, a similar risk should be considered possible until such studies are performed.

3.4 STUDY CALENDAR

1 cycle =21 days

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

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

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

Procedure	Screening	Cycle 1					Cycles 2 - 4					End of Treatment Visit (Cycle 4, Day 21)	Follow-Up Visit (6-8 weeks after completion of study therapy)[13]	Long-Term Follow Up (Optional)[14]
		Day 1	Day 3	Day 5	Day 8	Day 15	Day 1	Day 3	Day 5	Day 8	Day 15			
LMB-100		X	X	X			X	X	X					
SEL-110		X					X							
History and PE	X	X	X	X	X		X	X	X	X		X	X	
Weight		X	X	X	X		X	X	X	C2 only		X	X	
Height		X												
Vital signs[1]	X	X	X	X	X		X	X	X	C2 only		X	X	
Performance Score	X	X					X							
Labs[2]	X	X	X	X	X	X[3]	X	X	X	X	X[3]	X	X	X
Urinalysis	X	X					X							
HLA Typing (Class I and Class II)		X[4]												

Procedure	Screening	Cycle 1					Cycles 2 - 4					End of Treatment Visit (Cycle 4, Day 21)	Follow-Up Visit (6-8 weeks after completion of study therapy)[13]	Long-Term Follow Up (Optional)[14]
		Day 1	Day 3	Day 5	Day 8	Day 15	Day 1	Day 3	Day 5	Day 8	Day 15			
Anti HIV, anti HCV, HBSAg	X													
Urine or serum hCG in women of childbearing potential[5]	X	X[6]					X						X	
Confirmation of dx[7]	X													
NIH Advance Directives Form		X[8]												
Biopsy (optional)		X[9]					C3 only[10]							
Correlative Research Studies		Please see section 5.2												
PK		Please see section 5.2												
CT CAP and/or MRI	X	Every 6 weeks ± 7 days										X[11]	X[11]	X
FDG-PET	X	Every 6 weeks ± 7 days										X[11]	X[11]	X
ECG[12]	X	X	X	X			X	X	X				X	
Echocardiogram	X													
Adverse Events							Monitored continuously							
Concomitant Medications							Monitored continuously							

[1] **At screening:** heart rate, blood pressure, body temperature, pulse oximetry. **During the infusions** of all study medication in Cycles 1 and 2, vital signs (heart rate, blood pressure, body temperature) have to be monitored pre-infusion and every 15 minutes (± 5 minutes) during the

infusion and then every 30 minutes (± 10 minutes) from the end of the infusion until the infusion line is removed. From Cycle 3 onwards, vital signs have to be monitored only pre and post infusion if study medication has been tolerated well in previous cycles.

[2] CBC with differential, Acute Care Panel, Hepatic Panel, Mineral Panel, Lipid Panel, creatine kinase, C-reactive protein, PT, PTT, fibrin degradation products. On day 1 of each cycle, labs for lactate dehydrogenase (LDH), uric acid and CA125 will also be drawn.

[3] Day 15 labs may be performed outside of NIH

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

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

[6] Only required if more than 14 days have passed since screening.

[7] May be performed at any time prior to enrollment. Please see section 2.2 for tissue requirements

[8] As indicated in section 10.3, all subjects will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

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

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

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

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

[13] The assessments listed refer to those that will be performed if the patient is seen in clinic **6- 8 weeks after the last dose of study drug**. If the patient is unable to return to the clinic for the follow up visit, an adverse event assessment will be performed by telephone.

[14] Study visits after safety follow up visit will be encouraged but not required. Only patients who are removed from study therapy for reasons other than disease progression will be invited (approximately every 6 weeks) for the indicated scans and labs until disease progression.

3.5 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.

3.5.1 Criteria for removal from protocol therapy

- Progressive disease
- Participant has completed 4 cycles of study therapy
- Participant requests to be withdrawn from active therapy
- Positive pregnancy test
- Unacceptable Toxicity as defined in sections 3.1.1 and 3.3
- Requirement for any of the prohibited medications listed in section 4.1.2
- Investigator discretion

3.5.2 Off-Study Criteria

- Completed study follow-up period
- Decision to end the study
- Participant requests to be withdrawn from study
- Death

3.5.3 Off Protocol Therapy and Off-Study Procedure

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

4 CONCOMITANT MEDICATIONS/MEASURES

4.1.1 Permitted Therapy

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter (OTC) drugs, approved dietary and herbal supplements, nutritional supplements) used by a patient from the screening period until the follow-up visit. If any treatment is given within 4 weeks prior to screening this should be reported to the investigator and recorded in the eCRF.

All therapy and/or medication administered to manage adverse events should be recorded on the Adverse Event eCRF. Supportive care may be administered according to NIH CC Pharmacy Guidelines unless otherwise specified in section 3.3.

4.1.2 Prohibited Therapy

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

- Any other investigational therapy
- Cytotoxic chemotherapy agents other than study agents
- Radiotherapy. Note: palliative 8Gy radiotherapy is allowed.
- Strong inhibitors or inducers of CYP3A4 (as can be found here: Flockhart Table or in another frequently updated source)
- Drugs known to interact with Rapamune such as cyclosporine, diltiazem, erythromycin, ketoconazole (and other antifungals), nicardipine (and other calcium channel blockers), rifampin, verapamil
- Any medications or treatments that may adversely affect the immune system such as allergy injections, immune globulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids (oral or injectable)
- Other systemic anti-neoplastic agents and targeted therapies

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

All concomitant treatments must be documented in the eCRF.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 LMB-100 Pharmacokinetic Assessments

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

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

5.1.1.1 Sample collection:

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

5.1.1.2 Sample processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

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

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

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

5.1.1.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to the below address 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 SEL-110 Pharmacokinetic assessments

Whole blood levels of rapamycin (SEL-110) will be measured using a validated LC/MS method. All blood samples for PK assessment will be collected from an IV line different to that receiving the infusion to measure concentrations for SEL-110 for all patients. The date and time of each sample collection will be recorded. If multiple samples are drawn at a given time point, the PK sample should take precedence.

5.1.2.1 Sample collection:

Samples will be collected in 3.0 mL, K_3EDTA tubes (purple top) at the times defined in section 5.2. Samples should be inverted 8 after collection. NOTE: Do not shake or centrifuge samples.

Processing within 60 minutes of blood collection is highly preferred.

5.1.2.2 Sample Processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

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

1. Divide whole blood into two 4.0 mL cryovial (at least 1.5 mL in cryovial A and the rest in cryovial B).
2. Freeze upright at -80º C. The time between sample collections to placement in the freezer should not exceed 120 minutes

Do not Shake. Do not Centrifuge.

5.1.2.3 Sample Shipping

Do NOT ship sample A and B together. Sample B should remain stored on site at -80º until sample received report is received for sample A

Samples will be shipped by the CPP on dry ice along with sample manifest to Sannova Analytical Inc.

Malleswar Kollu.
Sannova Analytical Inc.
155 Pierce Street
Somerset, NJ 08873
Phone No: 732-560-0066
Fax: 732-560-0266

Please send shipment notification to: Malleswar Kollu (kollu@sannova.net), Shalini Pasuparthy (shalini@sannova.net), Pinakin Patel (pinakin@sannova.net)

5.1.2.4 Sample Storage

Samples will be stored in the CPP until shipment to Sannova Analytical Inc.

5.1.3 Assessment of LMB-100 anti-drug antibodies (ADAs)

5.1.3.1 Sample Collection

Samples will be collected before LMB-100 administration on days 1 and 5 of each cycle (See section 5.2). Sample may be collected at the same time as the anti-PEG sample referenced in section 5.1.4.

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

5.1.3.2 Sample Processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

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

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

Autoantibody levels will be retrospectively assessed.

5.1.3.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to the below address 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

5.1.3.4 Sample Storage

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

5.1.4 Assessment of anti-PEG antibodies

5.1.4.1 Sample Collection

Samples will be collected before SEL-110 administration on day 1 of each cycle in which SEL-110 is administered (See section 5.2)

Collect subject's venous blood contralaterally to the site of injection into a 5 mL SST Vacutainer. Invert and gently mix the SST Vacutainer 5 times. Place upright at room temperature until observe a clot (approximately 30 minutes).

5.1.4.2 Sample Processing

Samples will be processed in the Clinical Pharmacology Program.

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

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

Centrifuge within one hour of collection by spinning SST Vacutainer at 1100 g for 15 minutes in a centrifuge with swinging buckets or 1100 g for 15 minutes in a fixed angle centrifuge.

Remove from centrifuge and transfer plasma in approximately two equal volumes to two 2 mL cryovials, designated as primary sample A and sample B.

Immediately freeze the plasma samples in -80ºC freezer until shipped to TGA. Do NOT ship sample A and B together. Sample B should remain stored on site at -80ºC until sample receipt at TGA is confirmed for sample A.

5.1.4.3 Sample Shipping

Samples will be shipped by the CPP on dry ice to TGA Sciences Inc.

TGA Sciences, Inc.
Attn: Denise McSweeney (LMB)
47 Hall Street
Medford, MA 02155

5.1.4.4 Sample Storage

Samples will be stored in the CPP until shipment to TGA Sciences.

5.1.5 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.

5.1.5.1 Sample Collection

Samples will be obtained prior to the first LMB-100 dose of each cycle 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 labeled as follows:

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

5.1.5.2 Sample Processing

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

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.

5.1.5.3 Sample Storage

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

5.1.6 Retrospective Analysis of Mesothelin Expression in tumor tissue

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

5.1.6.1 Specimen collection

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

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

PE-based RITs cause dose-limiting CLS. At low doses CLS manifests as mild and transient weight gain, hypoalbuminemia, and peripheral or facial edema. At higher doses it can cause life-threatening cardiopulmonary compromise. Previous studies in rats have indicated that pathological changes indicative of CLS onset occur within just two hours of toxin administration and even when the PE fragment lacks a targeting domain.(38) In vitro studies with cultured endothelial cells have

demonstrated that super-physiologic doses of PE-based RITs cannot induce endothelial cell toxicity unless the cells express the RIT target.(39) Together these data suggest the hypothesis that **PE-based RITs cause CLS by triggering release of vasoactive cytokines by specific immune cells rather than through direct damage to endothelial cells**.

5.1.7.1 Specimen collection

To test this hypothesis, we will collect additional serum from participants in 4 mL SST tubes during each of LMB-100 therapy on:

- Days 1, 3, and 5 (Pre-dose)
- Day 8 (± 2 days)

5.1.7.2 Sample processing

Please e-mail Paula Carter pcartera@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 Paula Carter by e-mail or at 240-858-3191.

Cytokine levels will be retrospectively assessed.

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

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

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

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

Results obtained by this approach will be correlated with data obtained from immunohistochemistry of tumor biopsies as well as with data from analysis of peripheral blood populations, for a comprehensive study of the mechanism of action of LMB-100 in mesothelioma patients.

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

5.1.9 Tumor Microenvironment Studies Using Multiplex Staining Technologies

As referenced above, optional tumor biopsy will be performed in consenting patients when deemed feasible at the following time points: before initiation of therapy (archival tissue may also be used) and after two cycles of therapy. Portions of the tumor tissue collected at both timepoints and stored in the Hassan lab (see section 5.1.8) 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.

5.2 SAMPLE COLLECTION SCHEDULE

Unless otherwise indicated, samples are collected at the times given below. For the timed samples (PKs) samples should be collected within ± 5 minutes of given time.

Cycle	**Day**	**LMB-100 PKs** (5.1.1)	**SEL-110 PKs** (5.1.2)	**ADA** (5.1.3)	**Anti PEG antibodies** (5.1.4)	**Serum mesothelin and MPF** (5.1.5)	**PE mediated CLS (Cytokines)** (5.1.7)	**Tumor Sample**[a] (5.1.6, 5.1.8, 5.1.9)
		2 mL K_2EDTA tube (Times relative to start of LMB-100 infusion)	3mL K_3EDTA tube (Times relative to start of SEL-110 infusion)	2 mL K_2EDTA tube	5 mL SST Vacutainer	2 mL blood in 3.5 mL SST tube	4 mL SST tube	NA
Screening	Screening period ± 3 days							X[a]
1	1	--	Pre-dose	Pre-dose	Pre-dose	X	Pre-dose	X[b]
		--	0.5 hours					
		Pre-dose	1 hour					
		EOI	--					
		1 hour	2 hours					
		2 hours	3 hours					
		3 hours	4 hours					
		4 hours	5 hours					
		6 hours	7 hours					
		24 hours	25 hours					
	3		X				Pre-dose	
	5	--	X	Pre-dose			Pre-dose	
		--						
		Pre-dose						
		EOI						
		1 hour						
		2 hours						
		3 hours						
		4 hours						
		6 hours						
		24 hours						

Cycle	**Day**	**LMB-100 PKs** (5.1.1)	**SEL-110 PKs** (5.1.2)	**ADA** (5.1.3)	**Anti PEG antibodies** (5.1.4)	**Serum mesothelin and MPF** (5.1.5)	**PE mediated CLS (Cytokines)** (5.1.7)	**Tumor Sample**[a] (5.1.6, 5.1.8, 5.1.9)
		2 mL K_2EDTA tube (Times relative to start of LMB-100 infusion)	3mL K_3EDTA tube (Times relative to start of SEL-110 infusion)	2 mL K_2EDTA tube	5 mL SST Vacutainer	2 mL blood in 3.5 mL SST tube	4 mL SST tube	NA
	8		X[c]				X[c]	
2 -4	1	--	Pre-dose	Pre-dose	Pre-dose	X	Pre-dose	
		--	0.5 hours					
		Pre-dose	1 hour					
		EOI	--					
		1 hour	2 hours					
		2 hours	3 hours					
		3 hours	4 hours					
		4 hours	5 hours					
		6 hours	7 hours					
		24 hours	25 hours					
	3		X				Pre-dose	
	5	Pre-dose, EOI	X	Pre-dose			Pre-dose	
	8		X[c]				X[c]	
At time of Progression				X	X			
End of Treatment						X		
Follow-up Visit				X	X			

ADA =anti-drug antibody; EOI =End of LMB-100 infusion; PK =pharmacokinetic; MDSC= myeloid derived suppressor cell

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

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

c. Performed on day 8 ± 2 days

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 IRB notification and an executed MTA

5.3.1 Clinical Pharmacology Program

Upon arrival in the Clinical Pharmacology Program (CPP), OCD, CCR, NCI, all samples are barcoded, with data entered and stored in Patient Sample Data Management System (PSDMS), also known as *Labrador*, the system utilized by the CPP. This is a secure program, with access to the PSDMS system limited to defined CPP personnel, who are issued individual user accounts. PSDMS creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDMS 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. There are patient demographics that can be obtained to correlate with the samples through PSDMS. For each sample, there are notes associated with processing method (delay in sample processing, storage conditions on the ward, etc.)

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20°C or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Samples will be stored until requested by the researcher assigned to the protocol. All requests are monitored and tracked in PSDMS. 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 IRB approved protocol) and that any unused samples must be returned to the CPP.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

5.3.2 Leidos Biomedical, Inc. Lab

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

All cryopreserved samples are tracked for freezer location and storage criteria. All Samples are stored in a locked freezer at -70°C according to stability requirements. These freezers are located offsite at NCI-Frederick, at the Leidos Biomedical, Inc. Lab in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All use and requests for use will be recorded by the Leidos Biomedical, Inc. Lab. Any unused samples must be returned.

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

At the 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, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted. Otherwise, access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If specimens are to be discarded at any point, they will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

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

5.3.3 Laboratory of Dr. Raffit Hassan

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

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

5.3.4 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 approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.3.5 CAT-I Laboratory

The CAT-I laboratory (CAT-I) will obtain deidentified 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 report any loss or destruction of samples to the NCI IRB as soon as he/she is made aware of such loss. The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors such as a broken freezer or lack of dry ice in a shipping container, or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples, or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for a minimum of 30 days after removal from study treatment or until off-study, whichever comes first. Adverse events occurring more than 30 days after the last dose of study therapy are only required to be recorded only if they are considered to be serious and related to the investigational agent/intervention.

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, the IRB will be notified.

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:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

- Data will be shared through*:*
- 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 for response every 6 weeks (2 cycles). In addition to a baseline scan, confirmatory scans should also be obtained no less than 4 weeks following initial documentation of objective response.

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

For peritoneal mesothelioma, the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)(40) 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)(41) should be used as described in section 6.3.2.

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.(42-44) 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.(45)

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-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.(41) 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 MPM

Target lesion:

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

Evaluation of target lesions

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

6.3.2.2 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 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with

the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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 drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.

2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

    NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

Starting from the initiation of study therapy and continuing through the first 30 days after the subject receives the last administration of the investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For events that occur more than 30 days after the last administration of investigational agent/intervention, only report serious adverse events that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium***

and perinatal conditions - Other (pregnancy)" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 90 days after the last dose of LMB-100.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 90 days after the last dose should, if possible, be followed up and documented.

7.4 Safety Reporting Criteria to the Pharmaceutical Collaborators

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

All reports sent to the FDA will be sent concurrently to Selecta via email to Lloyd Johnston and Skip Sands: (LJohnston@selectabio.com and SSands@selectabio.com)

7.5 Data and Safety Monitoring Plan

7.5.1 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 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. Adverse events will be reported as required above. Any safety concerns,

new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

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

7.5.2 Sponsor Monitoring Plan

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

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

The monitoring program also extends to multi-site research when the CCR is the coordinating center.
This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.5.3 Safety Monitoring Committee (SMC)

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

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL HYPOTHESES

8.1.1 Primary endpoints:

The primary objective is to assess the safety and tolerability of LMB-100 in combination with SEL-110. Safety of the agent will be assessed as described in section 8.4.4.

8.1.2 Secondary endpoints:

The secondary endpoints are whether patients will have detectable levels of LMB-100 in their blood during cycle 4 of treatment and to determine the effect of SEL-110 on LMB-100 pharmacokinetics and the objective response rate (CR+PR) of SEL-110 plus LMB-100.

8.2 SAMPLE SIZE DETERMINATION

The primary objective of this pilot trial is to assess the safety and tolerability of LMB-100 in combination with SEL-110.

An initial dose escalation phase will follow a modified 3 + 3 design as described in section **3.1.2**, originally planned to have LMB-100 at 140 mcg/kg along with SEL-110 at 0.15 mg/kg and then 0.30 mg/kg (dose levels 1 and 2 respectively). As it was determined that the LMB-100 dose at DL1 caused unacceptable toxicity, the option to enroll patients at dose level -1 with LMB-100 at 100 mcg/kg was exercised. As of Amendment C, dose level -1A was also added for possible exploration if toxicity acceptable at dose level -1 with LMB-100 at 100 mcg/kg and SEL-110 at 0 0.30 mg/kg. As only 3 subjects were enrolled at dose level 1 before de-escalation to dose level -1, this dose escalation phase should require no more than 12 to 15 evaluable patients to complete. (3 [DL1]+ 3 or 6 [DL-1]+up to 6 [DL-1A]+0 [DL2]).

Once 6 patients have been safely treated at a single level (0-1 with DLTs at one of the dose levels explored), an additional 6 patients will be enrolled at that dose level. These 12 patients at the MTD will be evaluated for safety as described below.

The dose escalation phase may require up to 15 patients, and since 6 of these are expected to have initially been enrolled in the efficacy evaluation, only 6 additional patients are expected to be required solely for the secondary efficacy evaluation. Thus, it is expected that up to 21 patients may be needed to conduct the safety and efficacy evaluations. It is anticipated that 8-10 patients per year may enroll onto this trial. Thus, it is expected that accrual may be completed in approximately 2 – 2.5 years. To allow for the possibility of needing to replace patients who are inevaluable for the efficacy evaluation, the accrual ceiling will be set at 23 patients.

8.3 POPULATIONS FOR ANALYSIS

Evaluable for toxicity: All patients will be evaluable for toxicity after receiving at least one dose of each study agent (LMB-100 and SEL-110)

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

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

Evaluable for objective response: Only those patients who have measurable disease present at baseline, and have received at least one dose of therapy.

8.4 STATISTICAL ANALYSES

8.4.1 General approach

Safety of the agent will be assessed by reporting the grade of adverse events noted in each patient, and reporting the fraction with grade 3 and grade 4 adverse events at each dose level.

Levels of LMB-100 in the blood will be measured in cycle 4. The number of patients will be reported, and the fraction with detectable levels will be reported along with a 95% confidence interval. The effect of SEL-110 on LMB-100 pharmacokinetics and the anti-tumor efficacy of SEL-110 plus LMB-100 will also be reported.

8.4.2 Analysis of the primary endpoints

The primary objective is to assess the safety and tolerability of LMB-100 in combination with SEL-110. The safety of the agent will be assessed as described in section 8.4.4.

8.4.3 Analysis of the secondary endpoints

The six patients at the MTD will be evaluated for LMB-100 blood levels in cycle 4, along with 6 additional evaluable patients who will be enrolled at the same dose level to obtain the LMB-100 levels in 12 evaluable patients treated at a single dose level. If any of those 6 patients who were initially enrolled in the dose escalation phase are ineligible for the efficacy evaluation, they will be replaced to arrive at 12 patients who are evaluable for efficacy.

The patients will be identified as having a detectable level of LMB-100 or not. The fraction of patients who have a detectable level in their blood will be determined and this fraction will be reported along with a 95% confidence interval. Obtaining 6 or more patients out of 12 with detectable levels of LMB-100 would be considered a desirable outcome and will be reported as such.

Currently, approximately 60% of patients who are treated with LMB-100 have adequate blood levels of the treatment up through cycle 2 of treatment, but none have adequate blood levels in cycles 3 and 4. The goal will be to determine if the combination of LMB-100 and SEL-110 can result in 50% or greater of patients having measurable levels of LMB-100 in cycle 4.

If 12 evaluable patients are enrolled into the efficacy evaluation portion, the following are the probabilities of obtaining 6 or more patients with detectable blood levels of LMB-100 as a function of the true probability of having a detectable blood level:

True prob. of detectable LMB-100	Prob. of 6-12 out of 12 with detectable LMB-100
0.70	0.96
0.60	0.84
0.40	0.33

0.30	0.12
0.20	0.02

Thus, if the true probability of having detectable LMB-100 levels is 60% or greater, there is an 84% probability or greater of having 6 or more out of 12 patients with detectable levels, while if the true probability of having detectable LMB-100 levels is 30% or less, the probability of observing 6 or more out of 12 patients with detectable levels is 12% or less. Thus, observing 6 or more out of 12 patients with detectable LMB-100 levels is more likely to be associated with a regimen that has a much higher probability of resulting in detectable LMB-100 levels vs. one with a lower probability of resulting in detectable LMB-100 levels. A 95% confidence interval will be formed about the observed proportion of patients who have detectable levels of LMB-100 in cycle 4.

Where feasible, the following noncompartmental (NCA) pharmacokinetic parameters will be generated: maximum concentration (C_{max}), half-life ($T_{½}$), clearance (C_L), volume of distribution (V_z), the area under the concentration time curve from 0 to the last measurable concentration [$AUC_{(0–t)}$] and from 0 to infinity [$AUC_{(0–\infty)}$], along with the percent extrapolated value (%Extrap). These will be reported using descriptive statistics primarily, while any statistical testing done will be exploratory in nature.

The anti-tumor efficacy of SEL-110 plus LMB-100 will be reported by determining the fraction of patients who experience a clinical response to the combination. The fraction of patients with a PR or CR will be reported out of the 12 evaluable patients treated at the final dose of LMB-100 + SEL-110, along with an 80% and a 95% two-sided confidence interval.

8.4.4 Safety Analyses

Safety of the agent will be assessed by reporting the grade of adverse events noted in each patient, and reporting the fraction with grade 3 and grade 4 adverse events at each dose level. Safety data will be presented in individual listings. Summaries will also be prepared. The safety data will consist of the reporting of all adverse events, vital signs, physical examination data, laboratory safety data and ECG data.

8.4.5 Baseline Descriptive Statistics

Limited demographic and clinical characteristics of all patients will be reported.

8.4.6 Planned interim analyses

None will be performed.

8.4.7 Subgroup analyses

None will be performed.

8.4.8 Tabulation of individual participant data

Toxicity data may be reported on a per-patient basis if adequate events are noted, or may be summarized.

9 COLLABORATIVE AGREEMENTS

9.1 COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

This study is performed under a CRADA #3157 between NCI and Selecta Biosciences, Inc.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

LMB-100 is a mesothelin-targeted cFP and has shown preclinical dose-dependent activity in monotherapy and/or combination in xenografts representing MSLN-positive indications (NSCLC, mesothelioma, triple negative breast cancer, gastric cancer, pancreas, ovarian, potentially other tumor indications). The rationale to evaluate LMB-100 with SEL-110 in advanced/metastatic mesothelioma is to preliminarily assess the effect of SEL-110 on the pharmacokinetics of LMB-100. All patients meeting the criteria listed in section 2.1 are eligible for enrollment.

10.2 PARTICIPATION OF CHILDREN

There are no dosing or adverse event data are currently available on the use of LMB-100 with SEL-110 in patients <18 years of age; therefore, children are excluded from this study.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 10.5), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

10.4.1 Risks from Study Drugs

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

The risks of the study include those associated with study agent as discussed in section 11.

10.4.2 Blood Collection

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

10.4.3 Image Guided Biopsy Collection

The risks of the research biopsies collected at baseline and after 2 cycles include pain, bleeding and infection at the biopsy site. In addition, as the biopsies may be collected under CT guidance, subjects in this study may be exposed to approximately 1.6 rem. This amount is below the guideline of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee.

10.5 Risks/Benefits Analysis

Patients with advanced and/or metastatic mesothelioma are in continuous need of improved therapy options. This is especially true for patients where no standard therapy exists such as the patient population that will be eligible for this trial. Preclinical data has demonstrated promising anti-tumor efficacy of LMB-100 in xenograft models in monotherapy and combination therapy. Laboratory studies have further demonstrated that SEL-110 inhibits the development of antibodies against LMB-100. Therefore, LMB-100 + SEL-110 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 mesothelioma patients, those able to consent and those who lose the capacity to do so during the course of the trial, outweigh the risks associated with the proposed entry-into-human trial with LMB-100 + SEL-110.

10.6 Consent Process and Documentation

The procedures and tests involved in this study and the associated risks, discomforts and benefits of these processes, will be carefully explained to the patient or the patient's parents or guardian if he/she is a child, and a signed informed consent document will be obtained prior to entry onto the study. Consent for the optional biopsies performed on this study will be obtained at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

10.6.1 Telephone consent and re-consent

Re-consent in all cases as well as initial consent for screening, but not for treatment may be obtained by telephone using the following procedure: The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator.

10.6.2 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 LMB-100 (IND # 136483)

11.1.1 Source

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

11.1.2 Toxicity

Information in this section is based on preclinical studies with LMB-100, and clinical studies of the cytolytic fusion protein SS1-P. Patients should receive a full dose of LMB-100 unless a DLT

and/or a treatment limiting toxicity is observed. In case of DLT and/or treatment limiting toxicities, treatment with LMB-100 will be stopped until resolution of toxicity to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities. A delay of LMB-100 administration for up to three weeks of the planned schedule will be acceptable to allow for resolution of toxicity to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities. If toxicity does not resolve to NCI CTCAE Grade ≤ 2 hematological toxicities or Grade ≤ 1 non-hematological toxicities and the patient is unable to resume treatment with LMB-100 after this time, no additional doses will be administered and the patient will be withdrawn from study treatment.

11.1.2.1 Infusion-Related Reactions and Hypersensitivity Including Anaphylaxis

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

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

11.1.2.2 Risk of Immunogenicity and Potential Safety Impact

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

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

11.1.2.3 Risk of Inflammatory Reactions to Serosal Membranes

LMB-100 administration may cause inflammatory reactions to serosal membranes including pleuritis, characterized by pleuritic chest pain, dyspnea, and hypoxia and pericarditis, characterized by precordial chest pain, congestive heart failure, hypotension, and uremia. Clinical trials with SS1P monotherapy have led to reversible pleuritis and pericarditis. Patients who develop

symptoms of serosal inflammation should be closely monitored and receive standard treatments which may include corticosteroids.

11.1.2.4 Risk of Vascular Leak Syndrome

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

11.1.2.5 Risk of Renal Toxicity

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

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

11.1.2.6 Injection Site Reactions

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

11.1.3 Formulation and preparation

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

11.1.4 Stability and Storage

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

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

11.1.5 Administration procedures

Please refer to section 3.2.2.

11.1.6 Incompatibilities

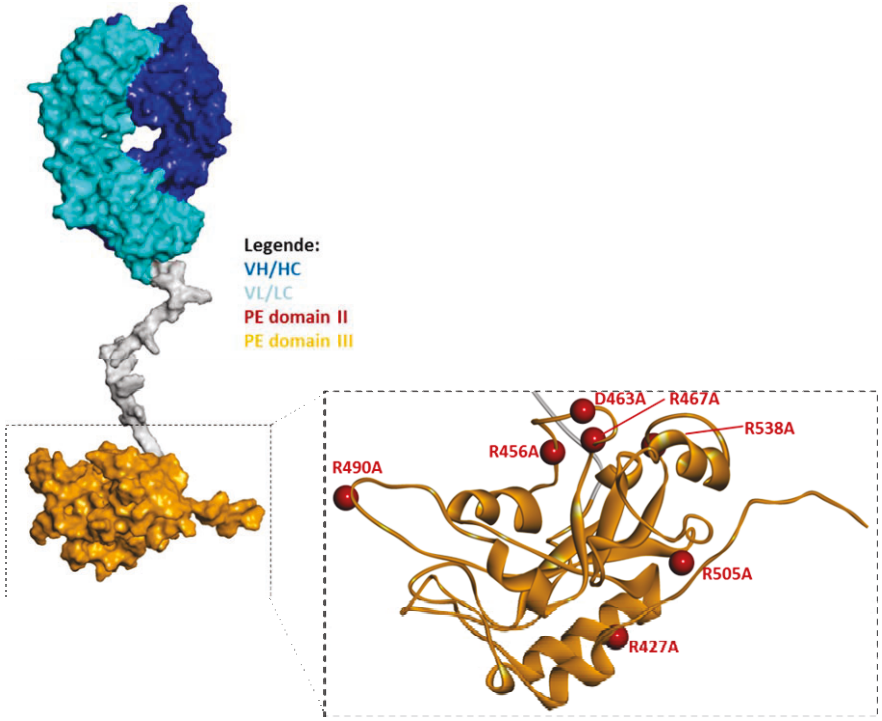
Pharmacodynamic drug interaction studies have not been conducted. LMB-100 is contraindicated in subjects with a history of severe allergic anaphylactic reactions to humanized, chimeric or mouse peptides/antibodies or to any components of the product.

11.1.6.1 Mechanism of action

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

11.1.6.2 Molecular Weight: approximately 73 kDa

11.1.6.3 Chemical Structure



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

11.2 SEL-110 (IND # 136483)

11.2.1 Source

SEL-110 is produced by Selecta Biosciences, Inc.

11.2.2 Toxicity

Consistent with ICH guidance, SEL-110 has been evaluated in nonclinical studies to support clinical study in humans, including safety pharmacology studies in rats and monkeys, as well as single and multiple dose toxicology studies in rats and monkeys. Immunotoxicology and local injection site reactions also were evaluated as a component of a repeat dose study in monkeys (Study 1933-004). Initial non-GLP dose finding studies of SEL-110 toxicity were completed in Sprague Dawley rats (Study 14-04312-N1) and cynomolgus monkeys (Study 14-04311-N1) in 8-week studies (given biweekly five times by IV bolus).

In the GLP repeated dose study of SEL-110 in rats (Study 1933-005), 1, 3 and 6 mg/kg were given as an intravenous slow bolus 3 times separated by 28 days. SEL-110 produced dose-dependent decreases in body weight and food consumption over the course of the study, primarily in the male animals that persisted for both sexes in the 6 mg/kg dose group through the end of the recovery period. Observation of reduced sizes of reproductive organs including testes and ovaries, as well as microscopic correlates, were observed at the 3 and 6 mg/kg dose levels. These changes are consistent with the known effect of rapamycin on the gonadotropin axis.(46) Microscopic changes were observed in the heart (mild cardiomyopathy) in males at the high dose 6.0 mg/kg group. At recovery, bilateral incipient cataracts were present in all male animals in the 6.0 mg/kg/dose group. Cataracts are a known toxicity of rapamycin in male rats, which is thought to be related to the development of hyperglycemia (Rapamune NDA 21-083). Based on the adverse findings observed at 3 and 6 mg/kg/dose including effects on the male reproductive system, 1 mg/kg/dose was identified as the No-Observed-Adverse-Effect-Level (NOAEL) for SEL-110 (rapamycin containing nanoparticle) when administered as a bolus intravenous injection on three occasions (Days 1, 29, and 57) in rats.

In the multi-dose study of SEL-110 in cynomolgus monkeys (Study 1933-006), 0.3, 1.0 and 3.0 mg/kg were given as an intravenous slow bolus 3 times separated by 28 days. SEL-110 was well tolerated at all doses, although there were observations of inappetence, vomitus and watery feces in males and females at the 1.0 and 3.0 mg/kg doses. Additionally, consistent with the immunosuppressive action of rapamycin, test article related microscopic findings of minimal follicular lymphoid depletion in the spleen in males and females at all dose levels and lymph nodes (mandibular and mesenteric) in males and females at 1.0 and 3.0 mg/kg were observed, but were not severe enough to be considered adverse. The no-observed-adverse-effect level (NOAEL) for this study was 3.0 mg/kg for males and females, the highest dose level tested.

Potential risks include those that have been identified in preclinical and clinical studies of SEL-110 and SEL-212 (combination of SEL-110 and pegylated uricase for the treatment of severe gout) as well as similar marketed products. These include 1) infusion reactions including anaphylaxis, 2) leukopenia and opportunistic infection, 3) metabolic disturbances (hyperglycemia, hypertriglyceridemia and hypercholesterolemia), 4) hypophosphatemia, 5) stomatitis, 6) oligo/azoospermia and 7) potential fetal development effects as seen in preclinical studies.

Rare cases of anaphylaxis were reported in the Phase 3 trials of Sirolimus (Rapamune®) during its development. Identification of the reason for these reactions has not been made whether it be the rapamycin or other ingredients in the oral formulation. Subjects with a history of anaphylaxis, angioedema or previous infusion reactions will be excluded from the trial.

Leukopenia has been reported in patients taking oral rapamycin. Whether the cause of the leukopenia is a direct relation to the rapamycin exposure, precedes or is due to the development of an opportunistic infection is unknown. Resolution of the leukopenia occurred in cases without subsequent infection with the stoppage of Sirolimus (Rapamycin) exposure. The current study will exclude subjects with any evidence of infection or developing infection by evaluating their WBC count and differential. Additionally, all subjects will be told to avoid anyone who has an active infection either bacterial or viral. Subjects will also have WBC evaluations performed during each treatment cycle per Study Calendar. In our single clinical study SEL-212/101 we have not seen this effect. (Data on file)

Metabolic disruptions (hyperglycemia, insulin resistance, hypertriglyceridemia and hypercholesterolemia) have been reported in post-transplant patients who have been exposed to rapamycin.(31, 32) The occurrence of post-transplant diabetes mellitus (PTDM) has been recognized for many years as a consequence of solid organ transplant immunosuppression.(33) Dyslipidemia is a well-recognized side effect of rapamycin therapy.(34) In our single ascending dose clinical study SEL-212/101 we have not seen this effect. (Data on file)

The exact mechanisms of rapamycin induced hyperglycemia, insulin resistance and its lipid effects are unclear at this time. It has been shown that rapamycin can diminish β-cell proliferation in hyperglycemic states thereby inhibiting the natural cellular response of neogenesis, proliferation, hypertrophy and a reduction in apoptosis.(35) Additionally, rapamycin has been shown to inhibit phosphorylation of IRS-1 and IRS-2 in human adipocytes mimicking changes seen in type 2 diabetes and in human peripheral blood monocytes associated with insulin resistance.(36) Rapamycin has also been shown to increase basal lipolysis and reduce lipid storage.(36) Due to these, subjects who participate will have these specific laboratories (fasting glucose, triglyceride and cholesterol levels) monitored closely for significant changes during their participation. Subjects will be monitored during each treatment cycle per Study Calendar.

The mechanism of hypophosphatemia is not known, but it has been seen in patients that have received mTOR inhibitors such as rapamycin.(37) And so, subjects will be monitored weekly during each treatment.

The mechanism of stomatitis is not known, but it has been seen in patients that have received mTOR inhibitors such as rapamycin as early as 7 days after treatment and responds to locally applied Clobetasol cream.(37) And so, subjects will be monitored during each treatment cycle per Study Calendar

Oligo/azoospermia has been seen in some male renal transplant patients taking an oral form of SEL-110 (rapamycin) administered chronically on a daily or three times weekly basis for the prevention of transplant rejection when treated for a period greater than six months. The mechanism for this effect is not known. In those patients where oligo/azoospermia has been seen partial to full reversibility of sperm production following cessation of treatment with the oral form of rapamycin has been demonstrated. Male patients should be informed that their fertility may be affected temporarily or permanently after being given SEL-110.

In preclinical animal studies performed with an oral form of the drug to be administered in this study (rapamycin, which is the active substance in SEL-110) showed adverse effects when given to pregnant rats. These effects included fetal death, reduced fetal weights, and delays in the formation of bones. It is not known whether these effects would also occur humans, a similar risk should be considered possible until such studies are performed. Because of these potential adverse effects, all females who directly participate in this clinical study must be of no childbearing potential and all males with female partners with childbearing potential must agree to use effective contraception and agree to continue doing so for four months after study drug dosing. Childbearing potential is defined as: a) less than 6 weeks after surgical removal of ovaries, tubes with or without removal of the womb or (b) pre- or peri-menopausal (less than 24 months of being without a period naturally).

SEL-110 was tested alone in the clinical study SEL-212/101 in an ascending single dose and also in combination with SEL-037 (pegsiticase, pegylated uricase). In the SEL-110 alone cohorts, two SAEs occurred at the highest dose (0.5 mg/kg) tested in this protocol. Both SAEs (stomatitis) resolved without any long-term consequences. Stomatitis is a known adverse event associated with rapamycin, the active ingredient in SEL-110. Data for this trial has been submitted to IND 124184. Additionally, the components of SEL-110 have been in previous clinical trials. The nanoparticles primarily consist of the class of biodegradable lactide polymers, part of the family of poly(lactide-*co*-glycolide) (PLGA) biodegradable polymers such as those found in the Food and Drug Administration (FDA) approved absorbable sutures Vicryl® and Dexon® and FDA approved products Zoladex®, Risperdal® Consta®, and Vivitrol®. Rapamycin (sirolimus) is the active ingredient in Rapamune, an FDA approved product for the prevention of organ rejection in kidney transplant patients.

SEL-110 is currently being studied in combination with SEL-037 (pegsiticase, pegylated uricase) in an open label Phase 2 study where patients diagnosed with gout and hyperuricemia are given an IV infusion of SEL-110 followed immediately by an IV infusion of SEL-037 (SEL-212 combination drug). Cohorts included patients receiving SEL-037 alone, patients receiving 3 combination doses of SEL-110 plus SEL-037 each 28 days apart followed by two doses of SEL-037 alone each 28 days apart and patients receiving 5 combination doses of SEL-110 plus SEL-037 each 28 days apart In this study SEL-110 doses of 0.05, 0.08, 0.1, 0.125, and 0.15 mg/kg have been studied.

Serious Adverse events in this study have primarily been related to infusion reactions to SEL-037 on repeat dosing in the control (no SEL-110 dose) or in the low dose SEL-110 plus SEL-037 arms.

One SAE infusion reaction occurred during infusion of SEL-110, which appears to be related to the rate of infusion being above the specified rate.

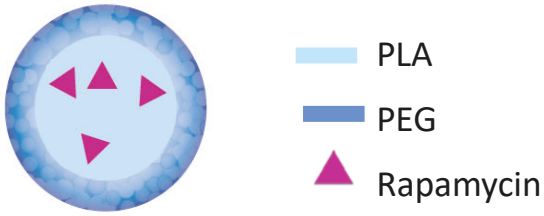
11.2.3 Formulation and preparation

11.2.3.1 Formulation

11.2.3.1.1 Drug Product Presentation

The SEL-110 nanoparticle is primarily composed of the biodegradable polymers PLA (poly(D,L-lactide) and PLA-PEG (poly(D,L-lactide)-block-poly(ethylene-glycol)). PLA as a formulation excipient is part of the broader PLGA (poly(lactide-co-glycolide)) family of biodegradable polymers that have more than 30 years of clinical use and are formulation components in a number of approved products, including Zoladex®, Risperdal® Consta®, Vivitrol® and Lupron Depot®. PEG has also been widely studied in clinical trials and is also a formulation component in many approved biological products, including macugen® and Krystexxa®.

Figure 15. Schematic of SEL-110 Nanoparticle



11.2.3.1.2 Composition

The SEL-110 drug product is a suspension of nanoparticles encapsulating rapamycin in phosphate buffered saline for intravenous administration. Table 14 lists the components of the SEL-110 drug product.

Table 14. SEL-110 Components

SEL-110 Component	**Description and Function**	**Nominal Formulation Content**
Rapamycin-containing nanoparticle	Drug substance carrier	2%
Phosphate Buffered Saline	Isotonic vehicle at neutral pH	98%

11.2.3.1.3 Investigational Drug Product

SEL-110 is supplied as a frozen nanoparticle drug product suspension.

SEL-110 is supplied as a frozen suspension of nanoparticles in PBS at a fill volume of 5 mL and a rapamycin content of 10 mg in a 10 mL Type 1 glass vial with a chlorobutyl stopper (gray, FluorTec® film on plug) and sealed with West 20 mm flip-off seals.

Table 15. Investigational Product

	Investigational Product
Product Name:	**SEL-110**
Dosage Form:	**Frozen nanoparticle suspension**
Unit Dose	**10 mg / 5 ml in the thawed state**
Route of Administration	**IV infusion**
Physical Description	**10 mL borosilicate glass vial with rubber stopper and aluminum-plastic combination cap**
Manufacturer	**Manufactured for Selecta Biosciences by Emergent BioSolutions**

11.2.3.2 Preparation

Each vial of SEL-110 will be allowed to thaw at room temperature and brought to room temperature over an approximate 2-hour period. Each vial will be inverted 20 times slowly (do not shake). Thawed SEL-110 should be administered as soon as possible, however is stable at room temperature and normal light conditions for 24 hours. Study drugs will be dosed on a mg/kg basis according to the cohort the subject is assigned. More than one vial may be required depending on the subject's weight and mg/kg dose.

11.2.4 Stability and Storage

Upon receipt of the SEL-110 vials at the clinical site, the vials should be stored in a secured way at -15 to -25 °C within the primary or secondary box container in order to protect the SEL-110 from long-term light exposure.

11.2.5 Administration procedures

Please refer to section 3.2.1.

11.2.6 Incompatibilities

Medications which are known CYP3A4 inhibitors or inducers incompatible with rapamycin. Patients taking medications that are known CYP3A4 inhibitors or inducers including natural products such as St. John's Wort or grapefruit juice may be included **ONLY** if they

discontinue the medication 14 days before dosing and are able to remain off the medication for the duration of the study.

Drugs known to interact with Rapamune such as cyclosporine, diltiazem, erythromycin, ketoconazole (and other antifungals), nicardipine (and other calcium channel blockers), rifampin, verapamil must be stopped at least 2 weeks prior to starting the trial and will not be used during the trial.

11.2.7 Mechanism of Action

SEL-110 drug product (DP) is a suspension of nanoparticles composed of PLA (poly(D,L-lactide)) and PLA-PEG (poly(D,L-lactide)-block-poly(ethylene-glycol)) encapsulating rapamycin in phosphate buffered saline for intravenous administration.

Rapamycin acts on mTOR and has been used as an immunosuppressive drug and is approved as a daily to thrice weekly oral tablet for the prevention of organ rejection in kidney transplant patients. By encapsulating the rapamycin in the biodegradable nanoparticle, the action of rapamycin is concentrated on dendritic cells and in preclinical models has shown to induce antigen specific tolerance as opposed to immunosuppression achieved by systemic administration of rapamycin.,

11.2.7.1 Physical Properties of SEL-110

Table 16. SEL-110 Physical and Chemical Properties

Physical/Chemical Properties	**Description**
Appearance	White to off-white semi-transparent suspension
pH	7.4 ± 0.5
Particle Size	Monomodal nanoparticle size distribution; mean size 150 nm

11.2.7.2 Chemical Properties

Chemical Abstract Service (CAS): 53123-88-9

IUPAC Name: (3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4]-oxaazacyclohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone

Company or Laboratory Code

Selecta Code: S-457

LSNE Code: BMTR0086

USAN Name: Sirolimus

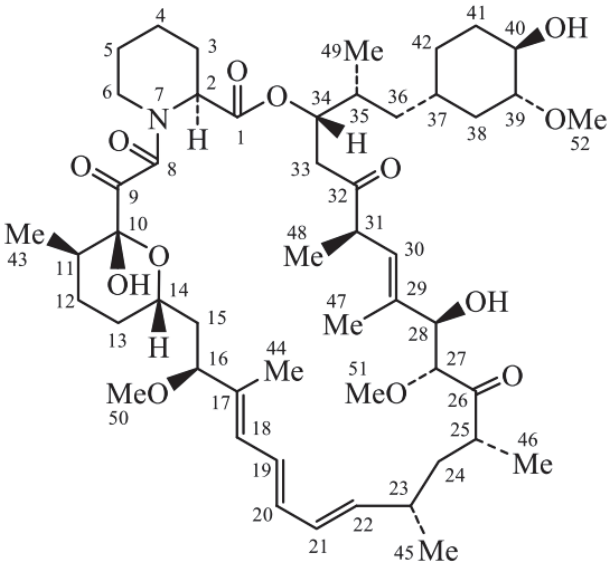
British Approved Name (BAN): Sirolimus

Other Non-Proprietary Names: Rapamycin, RAPA

11.2.7.3 Structural Formula

The active pharmaceutical ingredient in SEL-110 is rapamycin, also known as sirolimus, a molecule with a molecular formula of $C_{51}H_{79}NO_{13}$ and a molecular weight 941.19 Da. The structure of rapamycin is in Figure 16. Rapamycin is the active ingredient in Rapamune®, which is FDA approved for chronic daily oral administration for the prevention of organ rejection in kidney transplant patients.

Figure 16. Structure of Rapamycin



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13 APPENDICES

13.1 APPENDIX A-PERFORMANCE STATUS CRITERIA

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

13.2 APPENDIX B - COCKROFT-GAULT FORMULA

$$\text{Creatine Clearance} = \frac{(140 - \text{age}) \times \text{weight in kg}}{(\text{serum creatinine} \times 72)} \ (\times 0.85 \text{ for females})$$