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APPLICATION NUMBER:

125477Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125,477
Supporting document/s:	0
Applicant's letter date:	08/23/2013
CDER stamp date:	08/23/2013
Product:	Ramucirumab (CYRAMZA)
Indication:	Patients with gastric cancer and gastroesophageal junction adenocarcinoma after prior chemotherapy
Applicant:	Eli Lilly and Company 33 Imclone Drive Branchburg, NJ, 08876
Review Division:	Division of Hematology Oncology Toxicology (DHOT) Division of Drug Oncology Products 2 (DOP 2)
Reviewer:	G. Sachia Khasar, PhD.
Supervisor/Team Leader:	Whitney S. Helms, PhD.
Division Director:	John Leighton, PhD., DABT (DHOT) Patricia Keegan, MD. (DOP 2)
Project Manager:	Sharon Sickafuse

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125,477 are owned by [name of applicant] or are data for which Eli Lilly has obtained a written right of reference. Any information or data necessary for approval of BLA 125,477 that Eli Lilly does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125,477.

Pharmacology/Toxicology Labeling Review of Ramucirumab

The following table chronicles the labeling decisions in which the pharmacology/toxicology team had contributions for the initial approval of ramucirumab (CYRAMZA) for the treatment of patients with advanced gastric cancer or gastroesophageal junction adenocarcinoma.

The Applicant Proposed	FDA Recommends	Reasoning
(b) (4)	CYRAMZA™ is a human vascular endothelial growth factor receptor 2 (VEGFR2) antagonist indicated for the treatment of: Pregnancy: Based on its mechanism of action, CYRAMZA may cause fetal harm (8.1)	Established Pharmacologic Class (EPC): EPC was discussed with the product team and the pharm/tox associate director, Dr. Paul Brown. The EPC is based on the mechanism of action for ramucirumab. Use in pregnancy: label updated to reflect CFR
	8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Pregnancy Category C *Risk Summary* Based on its mechanism of action, CYRAMZA may cause fetal harm. Animal models link angiogenesis, VEGF and VEGF Receptor 2 to critical aspects of female reproduction, embryofetal development, and postnatal development. There are no adequate or well controlled studies of ramucirumab in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential hazard to a fetus. *Animal Data* No animal studies have been specifically conducted to evaluate the effect of ramucirumab on reproduction and fetal development. In mice, loss of the VEGFR2 gene	Label was updated using a hybrid approach while PLLR is being finalized. Specific literature based data was added to animal data section

(b) (4)	resulted in embryofetal death and these fetuses lacked organized blood vessels and blood islands in the yolk sac. In other models, VEGFR2 signaling was associated with development and maintenance of endometrial and placental vascular function, successful blastocyst implantation, maternal and feto-placental vascular differentiation, and development during early pregnancy in rodents and non-human primates. Disruption of VEGF signaling has also been associated with developmental anomalies including poor development of the cranial region, forelimbs, forebrain, heart, and blood vessels.	
	8.3 Nursing Mothers It is not known whether CYRAMZA is excreted in human milk. No studies have been conducted to assess CYRAMZA's impact on milk production or its presence in breast milk. Human IgG is excreted in human milk, but published data suggests that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Because many drugs are excreted in human milk and because of the potential risk for serious adverse reactions in nursing infants from ramucirumab, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.	Updated in accordance with CFR. P/T recommended the removal of "Human IgG is excreted in human milk, but published data suggests that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts" as this does not contribute a great deal to the decision about use of ramucirumab while breastfeeding; however, as the sentence appears in other labels for similar products and the Applicant felt it added value, the retention of this information was acceptable.
8.4 Pediatric Use Safety and effectiveness of CYRAMZA in pediatric patients have not been established. In animal studies, effects on epiphyseal growth plates were identified (b) (4)	**8.4 Pediatric Use** Safety and effectiveness of CYRAMZA in pediatric patients have not been established. In animal studies, effects on epiphyseal growth plates were identified. In cynomolgus monkeys,	Animal data concerning effects on bone growth that may be applicable to a pediatric population was moved here from section 13.2. This change is consistent with labels for other inhibitors of VEGF signaling

(b) (4)	anatomical pathology revealed adverse effects on the epiphyseal growth plate (thickening and osteochondropathy) at all doses tested (5-50 mg/kg). Ramucirumab exposure at the lowest weekly dose tested in the cynomolgus monkey was 0.2 times the exposure in humans at the recommended dose of ramucirumab as a single-agent.	
	8.8 Females and Males of Reproductive Potential Fertility Advise females of reproductive potential that CYRAMZA may impair fertility *[see Nonclinical Toxicology (13.1)]*. Contraception Based on its mechanism of action, CYRAMZA may cause fetal harm *[see Use in Specific Populations (8.1)]*. Advise females of reproductive potential to avoid getting pregnant while receiving CYRAMZA and for at least 3 months after the last dose of CYRAMZA.	New section of the label was added as proposed by PLLR. Data was based on the mechanism of ramucirumab as an inhibitor of VEGFR signaling and scientific findings on the effects of inhibition of VEGF signaling on fertility, the maintenance of pregnancy, and developmental problems in the fetus. Duration of contraception use in females was based on these risks and half-life of ramucirumab. No compelling information was available to suggest recommendations for males.
	12.1 Mechanism of Action Ramucirumab is a vascular endothelial growth factor receptor 2 (VEGFR2) antagonist that specifically binds VEGF Receptor 2 and blocks binding of VEGFR ligands, VEGF-A, VEGF-C, and VEGF-D. As a result, ramucirumab inhibits ligand-stimulated activation of VEGF Receptor 2, thereby inhibiting ligand-induced proliferation, and migration of human endothelial cells. Ramucirumab inhibited angiogenesis in an *in vivo* animal model.	Language was updated to reflect the pharmacology data supported by information submitted to the BLA. (b) (4) (b) (4)

13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** No animal studies have been performed to test ramucirumab for potential carcinogenicity, genotoxicity[redacted]	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** No animal studies have been performed to test ramucirumab for potential carcinogenicity or genotoxicity. Inhibition of VEGFR2 signaling in animal models was shown to result in changes to hormone levels critical for pregnancy, and, in monkeys, an increased duration of the follicular cycle. In a 39 week animal study, female monkeys treated with ramucirumab showed dose dependent increases in follicular mineralization of the ovary.	Additional data on effects of inhibiton of VEGF signaling on fertility and of ramucirumab on reproductive organs was added to the label as additional support for the recommendations in Section 8.8.
13.2 Animal Toxicology and/or Pharmacology [redacted] kidney (glomerulonephritis) occurred with doses of 16-50 mg/kg ([redacted] dose of ramucirumab as a single-agent). A single dose of ramucirumab [redacted] did not impair wound healing in monkeys using a full-thickness incisional model.	**13.2 Animal Toxicology and/or Pharmacology** Adverse effects in the kidney (glomerulonephritis) occurred with doses of 16-50 mg/kg (0.7-5.5 times the exposure in humans at the recommended dose of ramucirumab as a single-agent). A single dose of ramucirumab resulting in an exposure approximately 10 times the exposure in humans at the recommended dose of ramucirumab as a single-agent did not significantly impair wound healing in monkeys using a full-thickness incisional model.	[redacted]

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/s/

WHITNEY S HELMS
04/07/2014

MEMORANDUM

Cyramza (ramucirumab)

Date: April 7, 2014
To: File for BLA 125477
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting reviews for Cyramza conducted by Dr. Khasar, and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Cyramza may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
04/07/2014

MEMORANDUM

Date: February 21, 2014
From: Whitney S. Helms, Ph.D.
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for BLA # 125477
Cyramza (ramucirumab)
Re: Approvability of Pharmacology and Toxicology

On August 23, 2013 Eli Lilly and Company completed the submission of biological license application (BLA) 125477 for ramucirumab for the treatment of patients with advanced gastric cancer or gastroesophageal junction adenocarcinoma. Non-clinical studies examining the pharmacology and toxicology of ramucirumab provided to support BLA 125477 were reviewed in detail by G. Sachia Khasar, Ph.D. The findings of these studies are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Ramucirumab is a fully human IgG1 monoclonal antibody targeting the vascular endothelial growth receptor-2 (VEGFR2/KDR/FLK-1). Eli Lilly submitted pharmacology studies demonstrating that ramucirumab binds specifically to the human VEGFR2 receptor. Ramucirumab does not bind to other related human receptors or to the murine VEGFR2 receptor. Binding of ramucirumab to VEGFR2 was able to prevent binding of its endogenous ligand, VEGF, and to prevent ligand-induced phosphorylation of the receptor. Ramucirumab demonstrated anti-angiogenic activity in an *in vivo* model employing a mix of human endothelial cells and adipose derived stem cells implanted in mice and showed an ability to inhibit VEGF-induced proliferation and migration in *in vitro* systems using human cells. Because ramucirumab is unable to bind to murine VEGFR2, proof-of-concept studies were conducted in human tumor implanted mice using a surrogate antibody that recognizes the murine receptor. Administration of an anti-VEGFR2 antibody to these animals was able to inhibit the growth of an array of tumor types, including primary human gastric tumor fragments.

Consistent with the ICH S6 guidance, genetic toxicology studies were not conducted or required for ramucirumab. Carcinogenicity studies were not required to support the licensing application for a product to treat advanced human cancer and are neither planned nor expected as a post-marketing requirement at this time. Toxicology studies were conducted in the cynomolgus monkey. Anti-drug antibodies were common in treated animals, although exposure to ramucirumab in these studies appeared to be sufficient. The half-life in monkeys on Day 1 of both the 1 month and 39 week studies was approximately 4 days. Half-life in humans was reported to be longer—8 to 9 days. At the end of the 39-week study in monkeys, exposure to ramucirumab ranged from approximately 0.2-5.6 times the exposure after multiple doses in humans at the recommended dose of 8 mg/kg. The major toxicities noted in both general toxicology studies were changes to the epiphyseal growth plate in the femur; in treated animals in the 39 week study this toxicity consisted of epiphyseal thickening and osteochondropathy. Similar findings have been reported for other agents targeting VEGF signaling and may be relevant to pediatric populations. In the 39-week study the kidneys were also identified as a

target organ for toxicity, with findings of moderate to severe glomerulonephritis at dose levels at or above 16 mg/kg. This toxicity was only seen in animals at the 39 week timepoint suggesting that it is a late toxicity that could be seen with longer term administration of ramucirumab. Proteinuria has been noted across clinical trials in some patients treated with CYRAMZA. Marked subcutaneous hemorrhage was observed in one high dose group male that also had significant renal toxicity in the 39-week toxicology study. While the Applicant suggests that this finding is secondary to renal damage, due to the mechanism of action of ramucirumab as an inhibitor of angiogenesis, hemorrhage may have been exacerbated by the pharmacology of the drug.

The risk of delayed wound healing has been described following treatment with other inhibitors of VEGF signaling, thus, the Applicant conducted a dedicated study in monkeys specifically designed to investigate ramucirumab's effect on wound healing. At Day 4 following incision, 2 of the 8 wounds examined were fully closed in control animals versus no fully closed wounds at the high dose level (50 mg/kg), though all wounds had partial closure. By Day 8 no differences were seen between controls and treated animals. No clear differences in wound histopathology were noted between the groups. Overall, there was no definitive finding of significant effects on wound healing following single dose administration of ramucirumab.

No clear effects on blood pressure or cardiac electrophysiology were detected in monkeys at any dose level examined. Elevated levels of creatine kinase were present in both 1-month and 39-week toxicology studies. In the 1-month study, these increases correlated with findings of skeletal muscle degeneration. Creatine kinase can also be indicative of cardiac toxicity, though the only cardiac findings noted in monkeys were mononuclear inflammation and mononuclear aggregates detected histologically in the heart in both studies. Perivascular cuffing and mineralization of gray matter in the brain were also observed in some treated monkeys in the 39-week toxicology study, though there was no clear dose related response. Arterial thromboembolic events including myocardial infarction, cerebrovascular accident, and cerebral ischemia have been recorded clinically.

The requirement for reproductive toxicity studies was discussed by the Applicant prior to submission of the BLA. As non-human primates were the only relevant species for toxicological assessment of ramucirumab, in accordance with principles outlined in ICH guidances S9 and S6, the Agency agreed that an assessment of reproductive toxicology based on non-product specific literature might be acceptable in the case of ramucirumab. The Applicant provided literature describing investigations in rodents and monkeys demonstrating that VEGFR2 signaling is critical for the maintenance of pregnancy, that mouse fetuses lacking the receptor are not viable, and that complete or partial inhibition of VEGF, the ligand for VEGFR2, has teratogenic effects. Based on the information provided, disruption of this signaling pathway leads to both loss of pregnancy and fetal abnormalities; thus, the use of an antibody inhibiting this pathway during pregnancy represents a risk to the fetus. Ramucirumab is, therefore, not recommended for use during pregnancy unless the benefits to the mother outweigh the risks to a fetus. Nonclinical studies investigating the effects of ramucirumab on embryofetal development are not warranted at this time.

Recommendations: I concur with the conclusion of Dr. Khasar that the pharmacology and toxicology data support the approval of BLA 125477 for CYRAMZA. There are no outstanding nonclinical issues related to the approval of CYRAMZA for the treatment of patients with advanced gastric cancer and gastro-esophageal adenocarcinoma.

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/s/

WHITNEY S HELMS
02/21/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125,477
Supporting document/s:	0
Applicant's letter date:	08/23/2013
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Product:	Ramucirumab
Indication:	Patients with gastric cancer and gastroesophageal junction adenocarcinoma after prior chemotherapy
Applicant:	Eli Lilly and Company 33 Imclone Drive Branchburg, NJ, 08876
Review Divisions:	Division of Hematology Oncology Toxicology (DHOT) Division of Drug Oncology Products 2 (DOP 2)
Reviewer:	G. Sachia Khasar, PhD.
Supervisor/Team Leader:	Whitney S. Helms, PhD.
Division Directors:	John Leighton, PhD., DABT (DHOT) Patricia Keegan, MD. (DOP 2)
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1 Executive Summary

1.1 Introduction

Ramucirumab is a recombinant human immunoglobulin G, subclass 1 (IgG1) monoclonal antibody (mAb) against human vascular endothelial growth factor receptor 2 (VEGFR2), also known as human kinase domain-containing receptor, or KDR. VEGFR2 is one of the major transducers of VEGF signals in endothelial cells. Signaling through this receptor results in endothelial cell proliferation, migration, differentiation, and tube formation, critical processes in blood vessel development during vasculogenesis and the angiogenesis required for tumor vascularization. VEGFR2 signaling is also associated with increased vascular permeability and maintenance of vascular integrity. The critical role of angiogenesis in tumor development has been well-described and therapeutic agents targeting this process have shown efficacy in many tumor types. By targeting VEGFR2 specifically the Applicant hopes to maintain the efficacy of other anti-angiogenic therapies while reducing the toxicities associated with these therapies.

The Applicant is seeking a license to market ramucirumab for the treatment of adult patients with gastric cancers and gastroesophageal junction adenocarcinoma after prior chemotherapy. The proposed dose of ramucirumab is 8 mg/kg by intravenous infusion every two weeks.

1.2 Brief Discussion of Nonclinical Findings

The Applicant conducted a series of in vitro and in vivo studies to characterize ramucirumab. These studies demonstrated that ramucirumab binds to human VEGFR2 and can displace the VEGFR2 ligands, VEGF-A (IC_{50}=2.3 nM), VEGF-C (IC_{50}=0.7 nM) or VEGF-D (IC_{50}=0.3nM). In vitro assays demonstrated that binding of ramucirumab prevented the ligand-induced phosphorylation of the receptor as well as ligand-induced proliferation and migration. In addition, in an in vivo model of angiogenesis, using mice subcutaneously implanted with a mix of human endothelial progenitor cells and adipose-derived stem cells, ramucirumab demonstrated an antiangiogenic effect as measured by decreased hemoglobin and a reduction in vascular density in ramucirumab-treated mice compared to a control antibody. Since ramucirumab is not cross-reactive with mouse VEGFR2, its antitumor activity and anti-angiogenic mechanism of action could not be studied in the mouse. A mouse surrogate antibody, DC101, was developed for use in in vivo studies. In numerous in vivo tumor models, including systems using primary human gastric tumor fragments, inhibition of VEGFR2, either alone or in combination with other agents resulted in inhibition of tumor growth. The results of these studies support a mechanism of action for ramucirumab as a VEGFR2 specific inhibitor with anti-angiogenic potential.

Ramucirumab binds only to human and non-human primate VEGFR2, thus a single species was used for the toxicological assessment of the antibody. The Applicant submitted 5-week and 39-week GLP-compliant toxicity studies in cynomolgus monkeys.

In the 5-week study, ramucirumab was administered by intravenous infusion on Days 1, 15, 22, and 29 at dose levels of 0, 4, 12, and 40 mg/kg. In the 39-week study, ramucirumab was administered by intravenous infusion weekly at dose levels of 0, 5, 16, and 50 mg/kg. There were no consistent remarkable changes in hematologic parameters in either study. In the 5-week study high levels of creatine phosphokinase, though not dose-related, were suggestive of muscle injury and correlated with histopathologic findings of skeletal muscle degeneration. Elevated levels of creatine phosphokinase (CK) were also noted at the high dose level in the 39-week study, though without clear histopathologic findings of muscle damage. Elevations in CK are also possible signs of cardiac damage, though histopathological findings in the hearts of ramucirumab treated animals were limited to multifocal mononuclear aggregrates. There were no ramucirumab-related changes in ECG measurements or in blood pressure in either study, though hypertension is a clinical finding associated with ramucirumab treatment. Findings of mineralization and inflammation of gray matter as well as lymphocytic cuffing in the meninges and choroid plexus were noted as present in animals from all ramucirumab treatment groups. In tissue cross-reactivity studies, ramucirumab staining was observed in tissue slices from brains of both non-human primates and humans. Clinically, arterial thromboembolic events including cerebral ischemia have been associated with ramucirumab treatment.

Ramucirumab induced renal toxicity in the 39-week study. Moderate to severe glomerulonephritis occurred in male and female monkeys at ramucirumab doses ≥ 16 mg/kg. Glomerulonephritis was only observed at the end of the study on Day 273, not at the mid-term sacrifice on Day 85 suggesting that ramucirumab may have delayed effects on renal function. Increased findings of proteinuria have been reported in some clinical trials of ramucirumab.

Consistent with known effects of inhibition of VEGF signaling, treatment with ramucirumab caused alterations of epiphyseal growth plates in both the short and long term animal studies. Findings in the long term study consisted of thickening and osteochondrapathy (abnormal ossification with cartilage cell retention) at doses ≥ 5 mg/kg. Severity of these findings increased with increasing dose. While alterations in growth plates are unlikely to be of clinical significance in an adult population, they could be important if ramucirumab is used in a pediatric population.

Though gastrointestinal (GI) tract toxicity has been reported in some clinical trials of ramucirumab, there was little evidence of GI toxicity in the animal studies; GI findings were limited to histopatholgic signs of moderate inflammation of some sections of the GI tract in one animal. Severe subcutaneous hemorrhage in the skin, away from site of administration, was also reported in one high dose animal; while the Applicant suggests that hemorrhage at this site was related to severe renal toxicity in the animal, given the pharmacology of ramucirumab, bleeding may have been exacerbated due to the presence of the drug.

Based on the pharmacologic activity of the drug, FDA asked the Applicant to conduct a wound healing study. Administration of single dose of up to 50 mg/kg in monkeys did

not have a significant effect on histological findings following an incisional wound. On Day 4 following incision two of the 8 wounds examined were fully closed in control animals versus no fully closed wounds at the high dose level (50 mg/kg), though all wounds had partial closure. By Day 8 no differences were seen between controls and treated animals.

An increased incidence of follicular mineralization in the ovary was observed in female cynomolgus monkeys treated with ramucirumab for 39 weeks at dose levels ≥16 mg/kg. Together with literature reports associating inhibition of VEGFR2 with a decline in hormone levels and lengthening of the follicular phase, these findings suggest that female fertility is, at least temporarily, likely to be impacted by treatment with ramucirumab.

Dedicated reproductive toxicology studies examining the effects of ramucirumab administration to animals during pregnancy have not been conducted. Instead the Applicant submitted a literature based assessment of the potential effects of inhibition of VEGFR2 signaling during pregnancy. This assessment was completed in accordance with principles cited in the International Gudiances for Industry (ICH) S9: Nonclinical Evaluation for Anticancer Pharmaceuticals and S6(R1): Addendum to Preclinical Safety Evaluation of Biotechnoology-Derived Pharmaceuticals. Overall the scientific literature supports a critical role for VEGFR2 signaling in the maintenance of pregnancy and in embryonic vasculogenesis. Complete loss of the VEGFR2 receptor in knockout mice resulted in embryonic lethality by Gestational Day 9.5. These embryos showed defects in the development of hematopoietic and endothelial cells as well as absence of yolk-sac blood islands and organized blood vessels. Studies using anti-VEGFR2 antibodies in mice and monkeys demonstrated adverse effects of pathway inhibition on maternal ability to maintain pregnancy. As additional supportive data, complete or partial inhibition of VEGF, a ligand for VEGFR2, has been reported to result in impaired angiogenesis and blood-island formation, leading to severe developmental anomalies, including poorly developed and unsegmented branchial arches in the cranial region, unsegmented forelimb buds, significantly underdeveloped forebrain region, developmentally delayed common atrium and primitive ventricle in the heart region, rudimentary dorsal aortae, and markedly decreased thickness of the ventricular wall. Based on the data presented, disruption of this signaling pathway can lead to both loss of pregnancy and fetal abnormalities and use of an antibody inhibiting this pathway during pregnancy represents a risk to the fetus. Ramucirumab should, therefore, not be used during pregnancy unless the benefits to the mother outweight the risks to the fetus.

1.3 Recommendations

1.3.1 Approvability

The pharmacology and toxicology studies submitted are sufficient to support the use of ramucirumab in the proposed patient population. There are no outstanding

pharmacology/toxicology issues that would prevent the approval of ramucirumab, therefore, the pharmacology/toxicology team recommends the approval of this application.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Labeling recommendations will be addressed in a separate review.

2 Drug Information

2.1 Drug

CAS Registry Number: 947687-13-0

Generic Name: Ramucirumab

Code Name: IMC-1121B (ImClon); LY3009806 (Lilly)

Chemical Name

Immunoglobulin G1, anti-human vascular endothelial growth factor receptor (VEGFR)-2 extracellular domain (human monoclonal IMC-1121B γ-chain), disulfide with human monoclonal IMC-1121B κ-chain, dimer

Molecular Formula/Molecular Weight: 146.8 kDa

Structure

Ramucirumab is a human monoclonal antibody composed of 2 heavy chains (γ1-chains) molecules consisting of 446 amino acid residues each and 2 light chains (κ-chains) molecules consisting of 214 amino acid residues each. A schematic of the overall structure of ramucirumab is shown in Figure 1.

Pharmacologic Class: vascular endothelial growth factor receptor 2 (VEGFR2) directed antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 11856; (b) (4) (Letter of authorization submitted)

2.3 Drug Formulation

Ramucirumab Injection, Solution for Intravenous Infusion, 10 mg/mL, is a sterile solution, intended for single use. The drug product is provided in two presentations, 500 mg/50 mL and 100 mg/10 mL. The ramucirumab drug product is formulated in an (b) (4) solution at pH 6.0, containing 10 mM histidine (b) (4), 75 mM sodium chloride, 133 mM glycine, and (b) (4) polysorbate 80.

Unit Formula for Ramucirumab Drug Product, 500 mg/50 mL and 100 mg/10 mL

Ingredient	Quantity (mg/mL)	Function	Reference to Standards
Active Ingredient			
Ramucirumab	10	Active Ingredient	In-house
Other Ingredients		(b) (4)	
(b) (4) Histidine	0.65		USP, Ph.Eur, JP
Histidine Monohydrochloride	1.22		Ph.Eur, JP
Glycine	9.98		USP, Ph.Eur, JP
Sodium Chloride	4.38		USP, Ph.Eur, JP
Polysorbate 80	0.10		USP-NF, Ph.Eur, JP
Water for Injection	(b) (4)		USP, Ph.Eur, JP

(b) (4)

(Excerpted from the Applicant's submision)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Patients with gastric cancer and gastroesophageal junction adenocarcinoma after prior chemotherapy. The proposed dose of ramucirumab is 8 mg/kg every two weeks

2.7 Regulatory Background

Eli Lilly completed the submission of the BLA on August 23, 2013. The company had previous interactions with the Agency on the need to conduct animal studies to investigate reproductive toxicology in order to support the application under IND 11856. On June 29, 2012, the Applicant received a response to an information request regarding this subject informing them that in this case, FDA may accept an assessment of ramucirumab's developmental and reproductive toxicology that does not include ramucirumb-specific animal studies.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

In Vitro Pharmacodynamic Studies of Ramucirumab (IMC-1121B)

Study Number	Title
1001-09	Binding human VEGFR2
PT-1202	Binding to a panel of growth factor RTKs (Pan-Target Assay
IMC01	Binding to VEGFR1, VEGFR2, VEGFR-3
	Inhibition of VEGF-induced Endothelial cell proliferation: [*Lu et al. (2003)*]
	Inhibition of VEGF-induced mitogenesis: [*Zhu et al. (2003)*]
	Inhibition of VEGF-induced migration of leukemia cells: [*Zhu et al. (2003)*]
	Inhibition of VEGF-induced Calcium mobilization: [*Miao et al. (2006)*]
IMC04	Inhibition of VEGF-A, -C and -D binding to VEGFR2
	Inhibition of VEGF-C binding and activation of VEGFR2: [*Jimenez et al. (2005); Goldman et al.(2007); Tvorgov et al. (2010)*]
	VEGF-C-induced endothelial cell sprouting: [*Miao et al. (2006); Tvorgov et al. (2010)*]

IMC02	Analysis of Internalization of the IMC-1121B Antibody

In Vitro Pharmacodynamic Studies of DC101

Study Number	Title
1001-09	Binding to mouse VEGFR2
1001-09	Binding to mouse VEGFR2
	Binding to mouse VEGFR2: [*Rockwell et al. 1995*]
	Binding to mouse VEGFR1: [Luttun et al. 2002]
	Inhibition of VEGF-stimulated VEGFR2 phosphorylation: [*Rockwell et al. 1995*]
	Inhibition of VEGFR2 activation by VEGF-D: [Karnezis et al. 2012]

In vivo Studies

Study number	Title
1137-02	Efficacy of DC101 on HT-29 (p53 mutant) Xenografts
1245-02	DC101 Effects on Tumor Hypoxia in HT-29 Xenografts
1246-02	Efficacy comparison of KDR antibodies1121, 2C6 in HEL leukemia model
1255-02	Comparison of DC101 Efficacy on p53 w.t. vs. p53 Knockout HCT-116 Xenografts
1280-02	In vivo efficacy study of KDR antibodies (1121, 2C7 and 2C6) in HEL xenograft leukemia model
1282-02	VEGF Expression in HT-29 Tumor Bearing Mice
1302-02 (Repeat to include histology exam)	Comparison of DC101 Efficacy on p53 w.t. vs. p53 Knockout HCT-116 Xenografts
1304-02 (Repeat)	Efficacy of KDR antibodies in xenograft HEL Leukemia Model
1319-02	Histological Effects of anti-VEGF R2 mAb DC101 on p53-mutated HT-29 Xenografts
2221-03	DC101 Dose Response with HT -29 Xenografts
2212-03	Dose-dependent inhibition of BxPC-3 pancreatic tumor growth by DC101
3373-05	C225/A12/DC101 cocktail and C225/A12/3G3 biomarker studies in NCI-H460 Xenograft Model
3400-05	C225/A12/DC101 cocktail and C225/A12/3G3 biomarker studies in MDA-MB-435LM2 Xenograft Model
3460-05	MOA study of the combination of Erbitux and DC101 in GEO Xenografts
3493-05	Comparison of the antitumor efficacy of DC 101 and the combination of IMC-18F1 + MF1 in the MDA-MB-231 xenograft model
3549-05	C225/A12/DC101 cocktail and C225/A12/3G3 biomarker studies in NCI-H292 Xenograft Model
3553-05	C225, DC101 hypoxia study in GEO
3577-05	Anti-PDGFR-β 1B3 and anti-VEGFR2 DC101 Antibody Combination Therapy in NCI-H460 NSCL Carcinoma Xenografts (IV-466)
3608-06	VEGFR1, VEGFR2 combined inhibition in the GEO Xenograft Model
3643-06	Combination Therapy with Anti-VEGFR1, VEGFR2 mAbs in MX-1 Xenografts
3648-06	Non-histology MOA study of the combination of DC101, Cetuximab and IMC-A12 in GEO Xenografts
3730-06	Anti-PDGFR-β mAb 2C5 and C101 Combination in the HCT-8 Colon Carcinoma Xenograft Model; Efficacy of RON mAb 41A10 (IV-620)
3820-06	Antibody Combination 2C5+DC101 Therapy in NCI-H460 NSCLC Xenografts (IV-710)
3821-06	Antibody Combination 2C5+DC101 Therapy in HCT-116 Colorectal Carcinoma Xenografts (IV-711)
3888-07	Comparison of Sutent or DC101with or without Docetaxel in the MDA-MB-435LM2 Model
3892-07	Comparison of Sutent or DC101 with or without Docetaxel in the MX-1 Model

3913-07	2C5/DC101 MOA Study in NCI-H460 Xenografts (IV-805)
3964-07	Combination Therapy with anti-PDGF-Rβ 2C5 + anti-VEGFR2 DC101 + Gemcitabine in NCI-H292 NSCLC Xenografts (IV-857)
4028-07	Anti-PDGF-Rβ mAb 2C5 Combined with VEGFR2 mAb DC101 and Paclitaxel in NCI-H460 NSCLC Xenografts (IV-922)
4037-07	DC101 with or without Docetaxel Efficacy in an MDA-MB-231LP Metastatic Model
4087-07	DC101 with or without Sorafenib Efficacy In a SK-Hep1 Xenograft Model
4145-08	DC101 with or without Docetaxel Efficacy in an MDA-MB-231LP Metastatic Cancer Model
4260-08	MOA Study of DC1 01 + Sorafenib Combination In a SK-Hep1 Xenograft Model
4594-09	Efficacy of Sutent, DC101 and S12 in Combination with Docetaxel in an MDA-MB-231LP Metastatic Cancer Model
4597-09	Efficacy of DC1 01 in a Sorafenib-Refractory SC HuH-7 Hepatic Carcinoma Xenograft Model (IV-1500)
4615-09	mAb Combination IMC-18F1 + MF1 with mAb DC101 in an SK-HEP-1 Human Hepatic SC Xenograft Model (IV-1518)
4719-10	Efficacy of VEGFR mAbs in a Paclitaxel+S12-Refractory SC MDA-MB-231-LP-OS-PT Carcinoma Xenograft Model (IV-1622)
4721-10	VEGFR1-2 +5-FU/LV efficacy in a S12+Paclitaxei-Refractory DU4475 Breast Cancer Xenograft Model (IV-1624)
4723-10	Repeat-mAb Combination Study of [IMC-18F1 + MF1] with mAb DC101 in an SK-HEP-1 Human Hepatocellular Carcinoma SC Xenograft Model (IV-1626)
4850-10	[VEGFR1 or VEGFR2] mAb + IFL efficacy in an anti VEGF+Oxaliplatin Refractory HT-29 Colon Cancer Xenograft Model (IV-1753)
4985-10	VEGFR2 mAb DC101 ± IFL efficacy in an Anti-VEGF mAb + Oxaliplatin Insensitive HT-29 Colon Cancer Xenograft Model (IV-1888)
5086-11	Synergy analysis of the combination of IMC-11F8 and DC101 in a HCC827 human NSCLC xenograft model
5147-11	VEGFR2 mAb DC101 + IFL efficacy in an anti-VEGF mAb + oxaliplatin insensitive xenograft model of HT-29 human colon carcinoma (IV-2050)
5161-11	Colo205 human colon carcinoma tumor volume response to treatment with VEGFR2 mAb DC101 (IV-2064)
5211-11	Anti-VEGFR2 mAb DC101 with or without eribulin efficacy in an MDA-MB-231-LP-OS-PT human breast carcinoma xenograft model (IV-2114)
5213-11	DC101 MOA study in a HCC827 human NSCLC xenograft model
5310-12	Anti-VEGFR2 mAb DC101 with or without paclitaxel efficacy in an NCI-N87 human gastric carcinoma xenograft model (IV-2213)
5311-12	Anti-VEGFR2 mAb DC101 with or without paclitaxel efficacy in an MKN-45 human gastric carcinoma xenograft model (IV-2214)
5538-12	In Vivo Evaluation of DC101 as a Single Agent in the Treatment of Subcutaneous Gastric HuPrime Xenograft Models
IMC03	Inhibition of Angiogenesis by Human Endothelial Colony Forming Cells in a Co-Culture Model by LY3009806 (IMC-1121B): In Vivo Study in Mice
LLI-FTE-C01162010.S2.10	In Vivo Assessment of DC101 and S12 in the Treatment of Subcutaneous GAM046 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.12	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAF055 Gastric HuPrime Xenograft Model
LLI-FTE-CO1162010.S2.13	In Vivo Assessment of LL Y-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM11 0 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.14	In Vivo Assessment of LL Y-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM060 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.15	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM119 Gastric HuPrime Xenograft Model
LLI-FTE-	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the

C01162010.S2.16	Treatment of Subcutaneous GAF023 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.17	In Vivo Assessment of LL Y-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAF114 Gastric HuPrime Xenograft Model
LLI -FTE-C01162010.S2.18	In Vivo Assessment of LL Y-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM022 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.19	In Vivo Assessment of LL Y -31 C1, DC1 01 and S12 as Single Agents in the Treatment of Subcutaneous GAM025 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.21	In Vivo Assessment of LL Y -31 C1, DC1 01 and S12 as Single Agents in the Treatment of Subcutaneous GAM139 Gastric HuPrime Xenograft Model
LLI -FTE-C01162010.S2.23	In Vivo Assessment of LL Y-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM093 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.3	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM098 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.5	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM037 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.6	In Vivo Assessment of LLY-31C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAM019 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.7	In Vivo Assessment of IMC-31 C1, DC1 01 and 512 in the Treatment of Subcutaneous GAM016 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.8	In Vivo Assessment of LL Y-31 C1, DC1 01 and S12 as Single Agents in the Treatment of Subcutaneous GAF087 Gastric HuPrime Xenograft Model
LLI-FTE-C01162010.S2.9	In Vivo Assessment of DC1 01 and S12 as Single Agents in the Treatment of Subcutaneous GAM044 Gastric HuPrime Xenograft Model
1305-02	Efficacy Study of IMC-1121 in HL-60 Xenograft Leukemia Model

Pharmacokinetics

Study Number	Title
Report 2212-03	Dose-dependent inhibition of BxPC-3 pancreatic tumor growth by DC101
2034-03	DC101: Single-Dose Pharmacokinetics in Nude mouse
BDZ00030*	Ramucirumab (Process B and C): Single-Dose Pharmacokinetics Comparability in Cynomolgus monkey
20018776	Ramucirumab (Process C): Single-Dose Pharmacokinetics (Wound-Healing) in Cynomolgus monkey
SNBL023.04-SSR04014	Ramucirumab (Process A): Toxicokinetics (5 weeks – weekly dosing) in Cynomolgus monkey
1163-110	Ramucirumab (Process A): Toxicokinetics (39 weeks – weekly dosing) in Cynomolgus monkey

*A PK study in cynomolgus monkeys compared Ramucirumab drug products manufactured by two different processes, B and C. Ramucirumab from these two processes was formulated (b) (4) Process B material was formulated (b) (4) and was used in early clinical development. Process C material (also referred to as Process C0) was formulated (b) (4) and was used in Phase 3 clinical trials.

Distribution

The Applicant did not conduct tissue distribution studies with ramucirumab, stating that the drug is a monoclonal antibody and is expected to be largely confined to the extracellular space. This is supported by the relatively low volume of distribution determined in cynomolgus monkeys suggesting that ramucirumab is not extensively distributed outside of the vasculature. Similarly, metabolism studies were not performed with ramucirumab, as the catabolism of antibodies by mammalian systems is largely

understood, (degradation to small peptides and individual amino acids that are recycled) and formal studies of the metabolic degradation of these molecules are not warranted.

Toxicology

Study Number	Title
SNBL 023.04	A 5-week (4-dose) Repeat-Dose Toxicity Study of IMC-1121B in Cynomolgus Monkeys with a Terminal Sacrifice and a 6-week recovery Phase
1163-110	Thirty-Nine-Week Toxicity Study of IMC-1121B in Cynomolgus Monkeys
20018776	Single-Dose Wound Healing
IM1025	Tissue Cross-Reactivity

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The Applicant employed in vitro and in vivo studies to characterize the pharmacology of ramucirumab, including its binding and blocking activities at VEGFR2, and functional inhibition of VEGF/VEGFR2 signaling. The rodent surrogate, DC101 was used in numerous proof of concept xenograft studies. The Applicant also referenced literature on ramucirumab and DC101.

Study #: IMC01

ELISA Analysis of Purified IMC-1121B, IMC-3C5 and IMC-18F1 Antibody

This study was performed to assess the binding of purified IMC-1121B, purified IMC-3C5, and purified IMC-18F1 antibodies to their respective targets, vascular endothelial growth factor receptor 2 (VEGFR2/KDR/Flk1), vascular endothelial growth factor receptor-3 (VEGFR- 3/FLT4), and vascular endothelial growth factor receptor-1 (VEGFR1/FLT1), and to determine the specificity of all three ImClone antibodies to VEGFR where the antibodies act as negative controls for each other. (b) (4)

(b) (4)

Table 1: The 3 target proteins tested in ELISA analysis

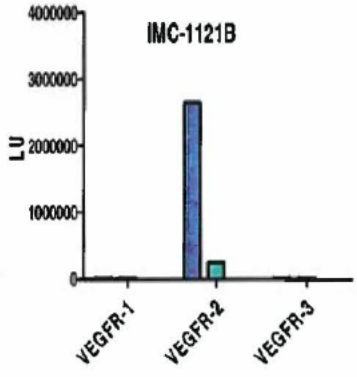
	Target Protein	Source	Catalog number	Lot number	Antibody target
1	VEGFR1 (flt1)	(b) (4)		YI9412031	IMC-18F1
2	VEGFR2 (KDR)			AZO1212081	IMC-1121B
3	VEGFR3 (flt4)			AIQ0512041	IMC-3C5

(Excerpted from Applicant's submission)

The graphical form of the results in Figure 2 show that IMC-1121B (middle panel) was specific for VEGFR2 There was no cross-binding to other target proteins by any of the antibodies.

Figure 2: Graphical representation of ELISA assay result

(b) (4)



(b) (4)

Bars represent luminence values of antibodies at the concentration s shown.

(Excerpted from the Applicant's submission)

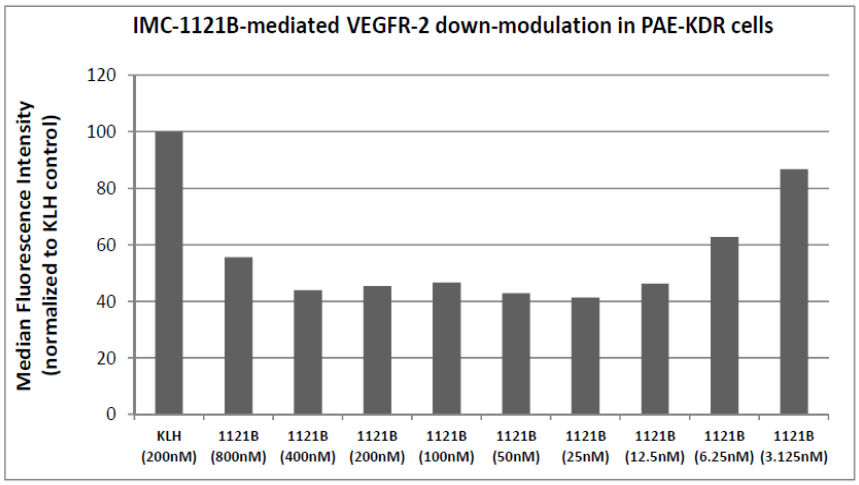
Study #: IMC02

Analysis of Internalization of the IMC-1121B Antibody

Receptor tyrosine kinases such as VEGFR2 are known to undergo internalization upon binding of their cognate ligands. This study investigated whether binding of the monoclonal antibody IMC-1121B to VEGFR2 on cultured endothelial cells leads to internalization of the receptor. Induction of the internalization by the ligand, VEGF165, was used as a positive control.

Cells were plated in 6-well dishes 1 to 3 days prior to treatment with either IMC-1121B or VEGF165 at the indicated concentrations at the start of treatment in culture media. The first experiments explored the possibility of IMC-1121B inducing internalization of VEGFR2 in PAE-KDR cells that were engineered to express high levels of VEGFR2. Cells treated with IMC-1121B for 24 hr demonstrated significant loss of surface receptors (Figure 3). High and very low concentrations of IMC-1121B were less effective than intermediate concentrations (between 25 and 400 nM), consistent with a mechanism which requires bivalent binding or crosslinking of VEGFR2 (ideally one antibody molecule per two receptor molecules) for optimal cell surface VEGFR2 down-modulation.

Figure 3: Internalization of VEGFR2 induced by IMC-1121B in PAE-KDR cells



(Excerpted from the Applicant's submission)

In a second set of experiments, human primary endothelial cells (ECs) were treated with VEGF165 for 24 hr or with ICM-1121B and then assayed for surface VEGFR2. Aortic endothelial cells (AEC) and microvascular endothelial cells (hMVEC) were used. Exposure of AEC or hMVEC, to VEGF165 led to dose dependent loss of surface VEGFR2. Similarly, in both AEC and hMVEC, increased receptor internalization was also observed following incubation with IMC-1121B. Receptor internalization following incubation with IMC-1121B increased slightly as the concentration of IMC-1121B was reduced.

The results of these studies suggest that exposure to IMC-1121B modulates VEGFR2 levels on the surface of EC in a concentration dependent manner.

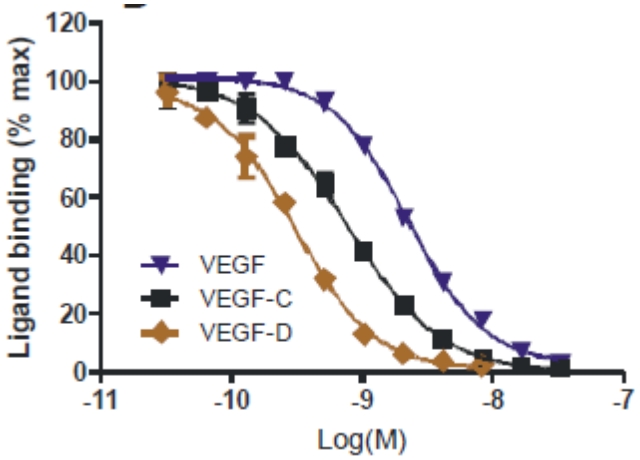
Study #: IMC04

Inhibition of Binding of VEGF-A, VEGF-C and VEGF-D to Human VEGFR2 by IMC-1121B

Although VEGF-A (VEGF) is the principal ligand for VEGFR2/KDR, human VEGFR2 can also be activated by two additional ligands, VEGF-C and VEGF-D, following maturation of these proteins by N and C terminal proteolytic cleavage. This study was performed to demonstrate that the human monoclonal antibody IMC-1121B inhibits binding of VEGF-A, -C and -D to VEGFR2/KDR/Flk1 in an in vitro (cell free) model.

IMC-1121B inhibited binding of VEGF (IC_{50} = 2.3 nM), VEGF-C (IC_{50} = 0.7 nM) and VEGF-D (IC_{50} = 0.3 nM) to soluble extracellular domain of human VEGFR2 in a dose-dependent manner (Figure 4). The binding was not inhibited by control antibody IMC-3C5 that is an antagonist of human VEGFR-3, the primary receptor for VEGF-C and VEGF-D. Range of IC_{50} values reflects the difference in affinities of various ligands for VEGFR2.

Figure 4: Inhibition of binding of VEGF (A), VEGF-C (B) and VEGF-D (C) to VEGFR2-Fc by IMC-1121B.



(Excerpted from the Applicant's submission)

Study #: IMC03

Inhibition of Angiogenesis by Human Endothelial Colony Forming Cells in a Co-Culture Model by LY3009806 (IMC-1121B): In Vivo Study in Mice

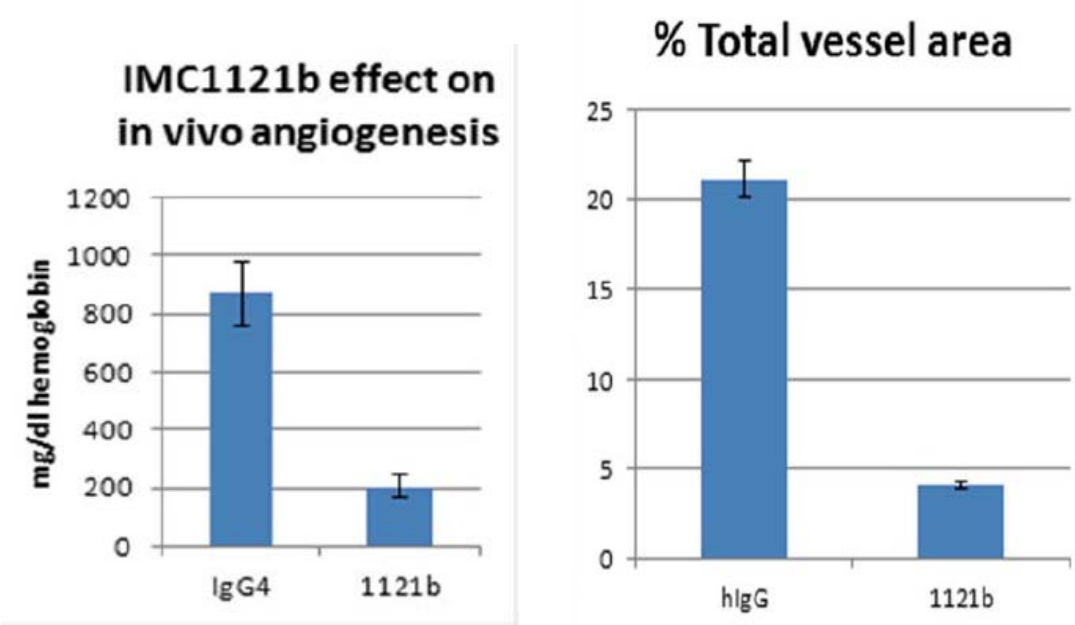
The objective of this study was to investigate evidence for anti-angiogenic activity of IMC-1121B in an in vivo model in mice.

Subcutaneous implantation of human endothelial progenitor cells and adipose-derived stem cells mixed with matrigel into female athymic nude mice (20-25 g; 10 mice per

group) leads to formation of a capillary network that forms anastomoses with dermal murine vasculature. The perfusion of this network was measured by quantifying the amount of hemoglobin in the matrigel plug. The vascularity of the plug was assessed by immunohistochemistry (IHC).

The ability of IMC-1121B to inhibit the formation human blood vessels in the matrigel plug compared to hIgG4 (control antibody) was tested. Antibody treatments were given to mice as a single intra-peritoneal dose of 10 mg/kg in a volume of 0.2 mL, five hours prior to implanting the matrigel plugs. IMC-1121B potently inhibited angiogenesis in the matrigel plug model as demonstrated by reduction in hemoglobin as well as reduction of vascular density by 81% (Figure 5).

Figure 5: Inhibition of angiogenesis by IMC-1121



(Excerpted from the Applicant's submission)

The results of this experiment show that IMC-1121B has anti-angiogenic activity in a mouse model of human blood vessel formation.

Study #: PT-1202

Pan Target ELISA Analysis of purified IMC-1121B Antibody

The pan target study was performed to assess the binding of purified IMC-1121B antibody to its target, vascular endothelial growth factor receptor [(VEGFR2), also known as kinase insert domain receptor (KDR) or fetal liver kinase 1 (Flk1)], and to determine if there is any cross reactivity with a panel of other proteins. The Pan Target assay is an in vitro sandwich based ELISA in which wells are coated with various target

proteins (Table 2), followed by challenge with a test antibody, and detection with a secondary anti-human kappa light chain horseradish peroxidase conjugated antibody. Controls included; a negative control (PBS or blocking solution alone) and a positive control (IMC-C225 specific for EGFR). Two experiments were run; 1 with the test antibodies diluted in PBS, the other with test antibodies diluted in 3% blocking solution.

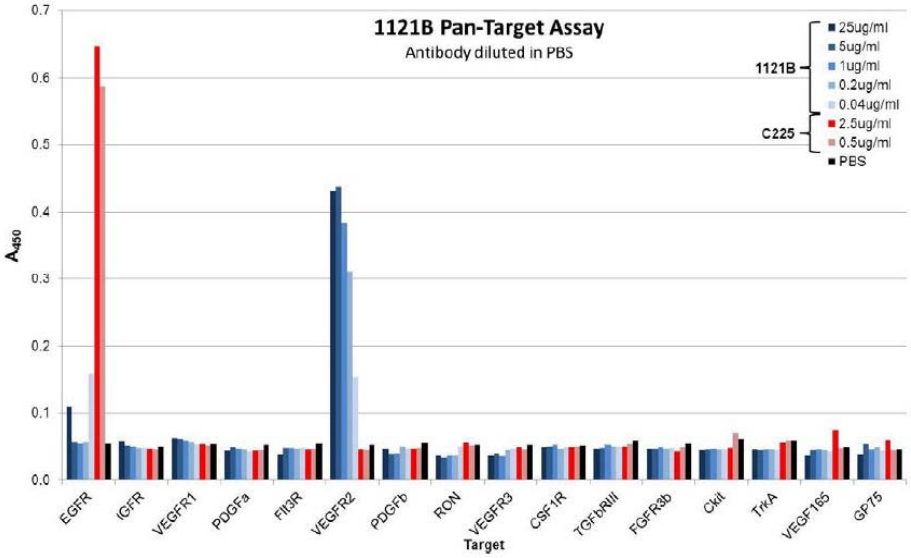
Table 2: 16 target proteins tested in a pan target ELISA.

	Target Protein	Source	Lot number	Antibody target
1	EGFR	ImClone Systems	04B0051	IMC-C225
2	IGF1R	ImClone Systems	60303	IMC-A12
3	VEGFR1 (flt1)	ImClone Systems	60110	IMC-18F1
4	PDGFα	ImClone Systems	70104	IMC-3G3
5	Flt3R	ImClone Systems	51102	IMC-EB10
6	VEGFR2 (KDR)	ImClone Systems	41227	IMC-1121B
7	PDGFβ	ImClone Systems	61221	IMC-2C5
8	RON (rMSPR)	(b) (4)	Lot # MNJ0407061	IMC-RON8
9	VEGFR3 (flt4)	ImClone Systems	61207	IMC-3C5
10	CSF1R	ImClone Systems	070131	IMC-CS4
11	TGFβRIII	ImClone Systems	90501	IMC-TR1
12	FGFR3b	ImClone Systems	NA	IMC-D11
13	ckit	(b) (4)	Lot # ABN1308031	IMC-CK6
14	TrkA	ImClone Systems	060825	IMC-TA1
15	VEGF165	(b) (4)	Lot #XQ1309081	D2
16	GP75	ImClone Systems	120725	IMC-20D7S

(Excerpted from the Applicant's submission)

The results of the experiment in which test antibodies were diluted in PBS, are shown in graphical form in Figure 6. The positive control, IMC-C225, was specific for its target, EGFR and the IMC-1121B was specific for VEGFR2, with no binding to other target proteins. The results of the experiment in which test antibodies were diluted in 3% blocking solution were similar to those using PBS.

Figure 6: Pan Target assay



Graphical representation of data from experiment using PBS during the antibody challenge step. Blue bars = dilution of IMC 1121B, Red bars = dilutions of C225 positive control, Black bar = 3% blocking solution control.

(Excerpted from the Applicant's submission)

The results of the pan target ELISA analysis of purified IMC-1121B (ramucirumab) demonstrate specific binding of IMC-1121B to VEGFR2.

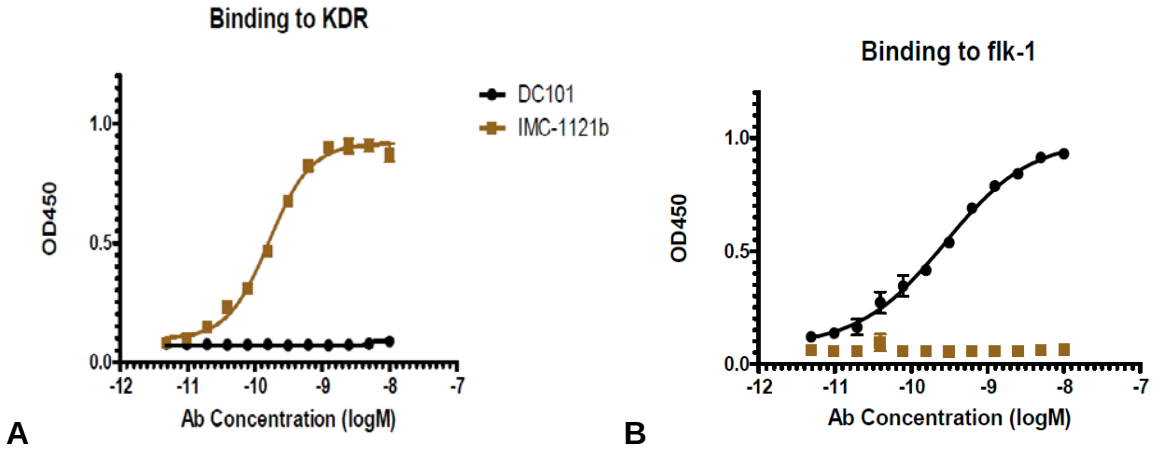
Report # 1001-09

Cross Binding Study of IMC-1121b and DC101 to Human Kinase Insert Domain-containing Receptor (KDR) and its Mouse Homologue Fetal Liver Kinase1 (Flk1) by ELISA and BIAcore (LY3009806)

This experiment evaluated the cross binding of anti-VEGFR2 antibody, ramucirumab (IMC-1121B), to the human extracellular domain of VEGFR2 (KDR) and its mouse homologue, fetal liver kinase1 (Flk-1) by ELISA and BIAcore.

As evaluated by direct binding ELISA, ramucirumab exhibited dose-dependent binding to the immobilized extracellular domain of human VEGFR2 with an EC_{50} of 0.16 nM （Figure 7A) and DC101 exhibited dose dependent binding to immobilized mouse Flk-1 with an EC_{50} of 0.28 nM (Figure 7B).

Figure 7: Dose-dependent binding of IMC-1121B to the human KDR (A) or DC101 to the mouse Flk-1 (B)



(Excerpted from the Applicant's submission)

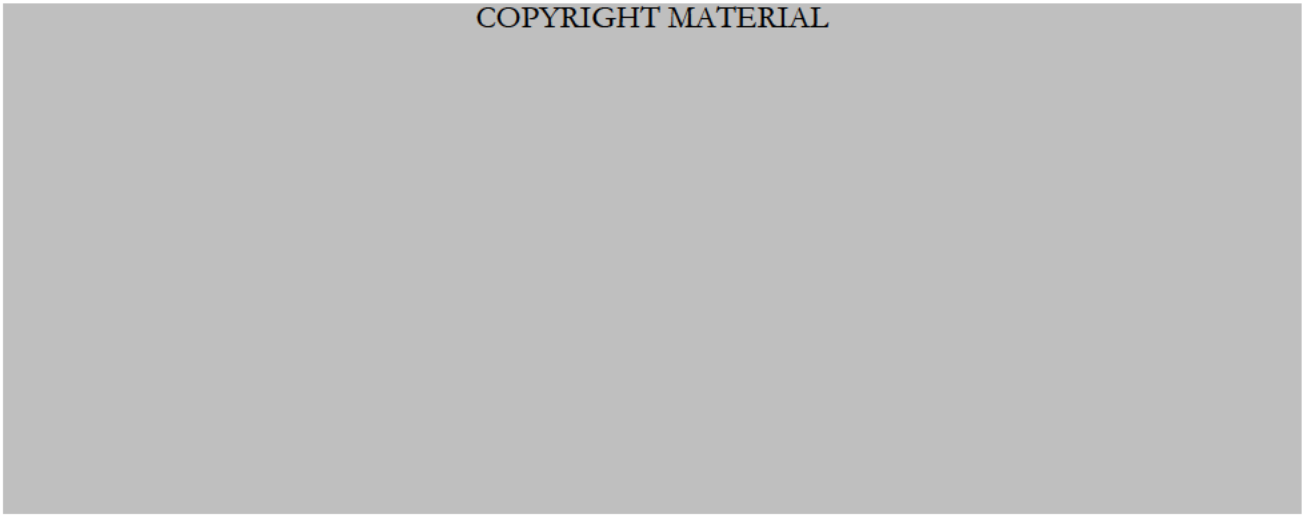
BIAcore binding assay results (data not shown) supported the finding of species specificity of VEGFR2 antibody binding between mouse and human proteins, indicating that, at 100 nM concentrations, ramucirumab, hVEGF (ligand for KDR) and mVEGF (ligand for Flk1) were able bind to immobilized VEGFR2, but DC101, the mouse anti-VEGFR2 antibody, could not, even at the higher concentration of 400 nM. Similarly, at 100 nM concentrations, DC101, hVEGF, and mVEGF were all able to bind to immobilized Flk1, but ramucirumab at 400 nM could not.

These results demonstrate the binding of IMC-1121B to the extracellular domain of human VEGFR2 but not to its mouse homologue Flk-1. In addition, the anti-murine Flk-1 antibody DC101 was able to bind to the murine Flk-1 but not to human VEGFR2. This study suggests that the mouse is not an appropriate model for the toxicological safety assessment of ramucirumab.

Lu et al, (2003)[1]

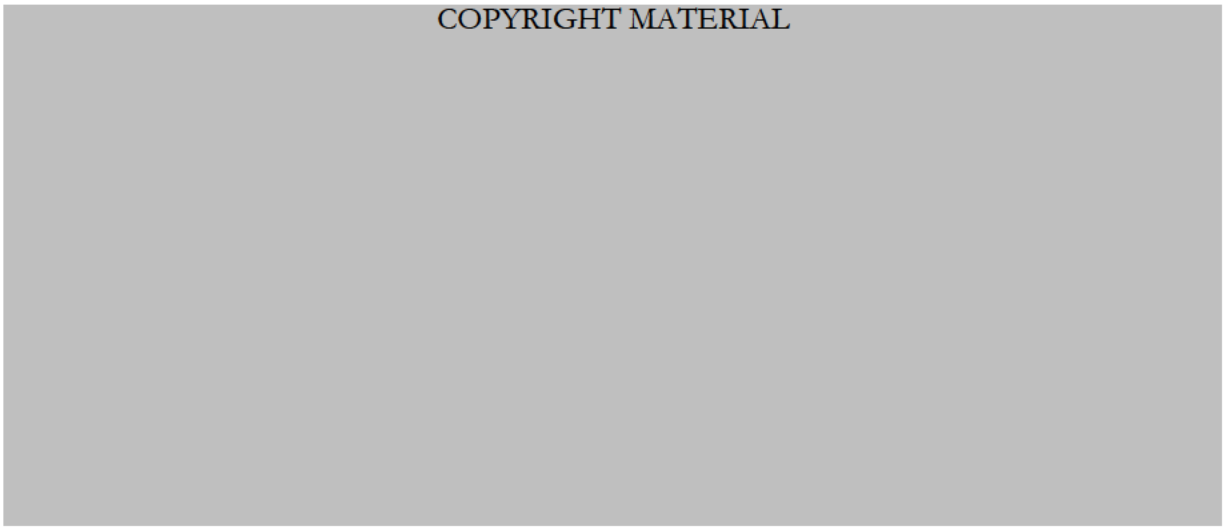
The Applicant submitted the paper by Lu et al, in further support of the pharmacological activity of ramucirumab. The authors demonstrated that both the ramucirumab (1121B) Fab fragment and the whole antibody were able to block binding of VEGFR2 to immobilized VEGF (Figure 8).

[1] Lu, D., et al (2003) *J Biol Chem* 278: 43496–43507



(Excerpted from Lu et. al.)

The authors went on to demonstrate that ramucirumab is able to inhibit the ligand induced phosphorylation of VEGFR2 in VEGFR2 expressing cells in a concentration dependent manner (Figure 9). In addition they showed that binding of the VEGFR2 by ramucirumab in the absence of VEGF does not result in receptor phosphorylation.

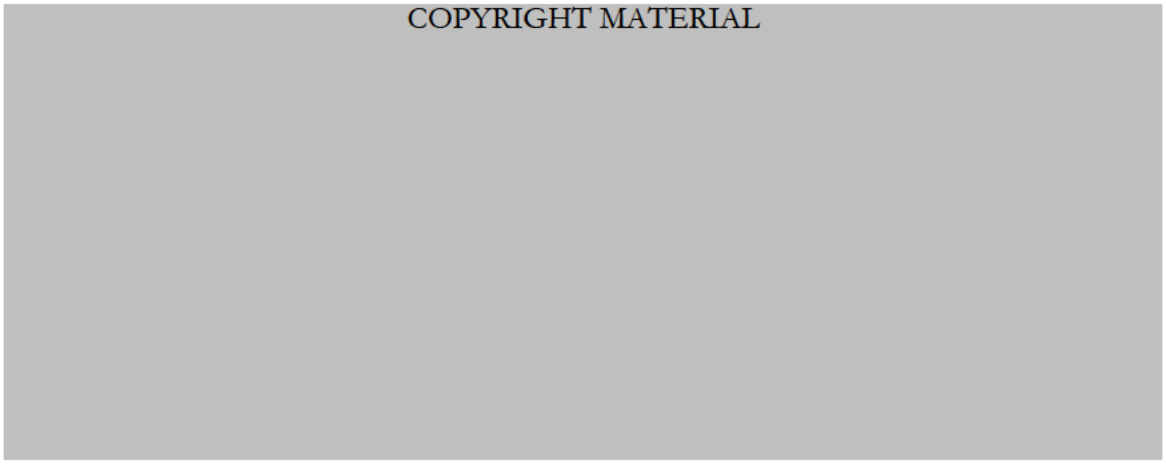


Zhu et al, (2003)[2]

The Applicant included the paper by Zhu et. al. as additional evidence of the pharmacological activity of ramucirumab in cell-based functional assays. The authors conducted experiments using human umbilical vein epithelial cells (HUVEC) that expressed VEGFR2. Radiolabelled VEGF was added to HUVEC cells in the presence of increasing amounts of ramucirumab or alternative anti-human VEGFR2 antibodies

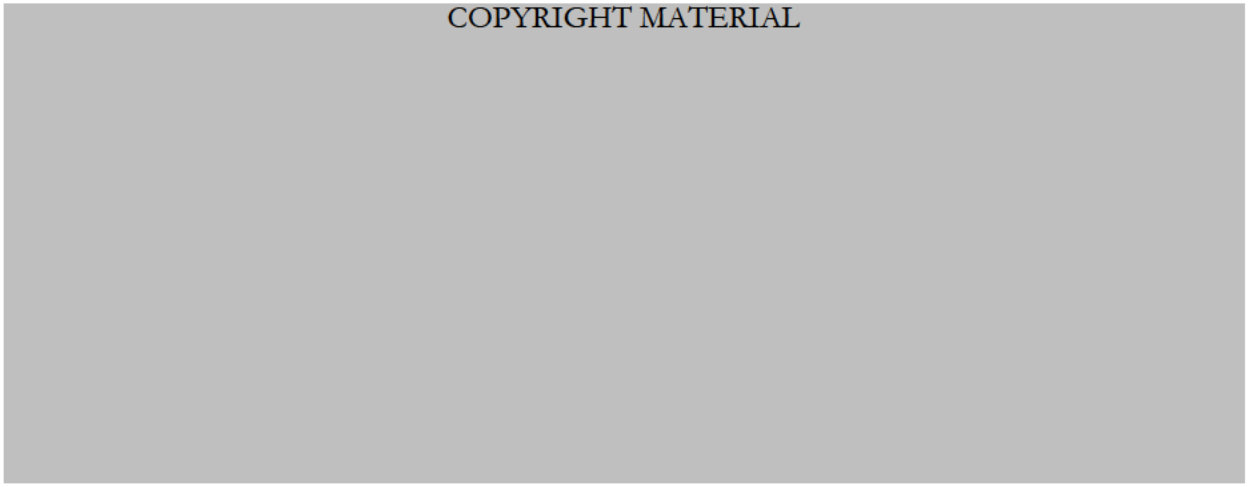
[2] Zhu Z., et al (2003) *Leukemia* 17:604-611.

(IMC-1C11 and 2C6). Cells were washed and then the amount of radiolabelled VEGF that remained bound was measured. Increasing amounts of anti-VEGFR2 antibodies, including ramucirumab were able to prevent VEGF binding to these cells. An irrelevant antibody control, IMC-C225, was included (Figure 10).



(Excerpted from Zhu et. al.)

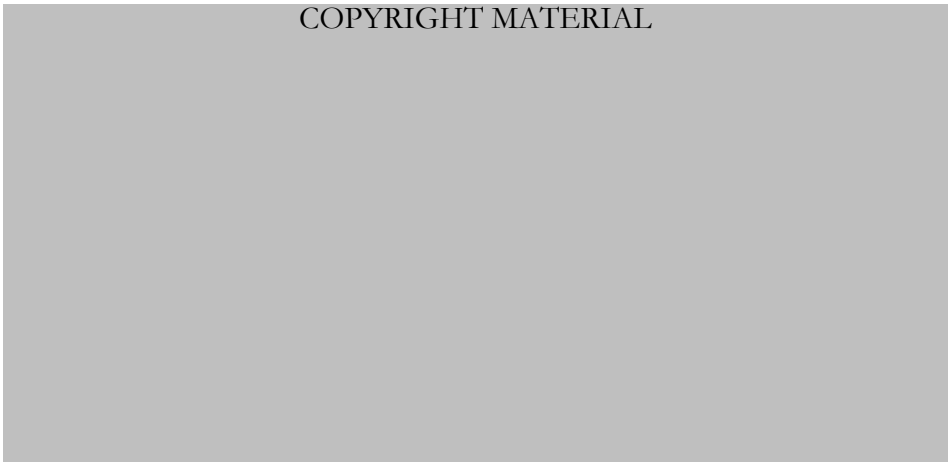
In addition, when VEGF was added to growth factor starved HUVEC cells that had been preincubated with increasing concentrations of ramucirumab or alternative anti-human VEGFR2 antibodies, there was inhibition of HUVEC cell proliferation in the presence of the antibodies. The IC_{50} of ramucirumab for HUVEC growth under these conditions was 0.7 nM (Figure 11).



(Excerpted from Zhu et. al.)

Finally, the authors demonstrated inhibition of VEGF-stimulated cell migration in the presence of ramucirumab using human leukemia cell lines shown to express VEGFR2. Cells were in transwell inserts in the presence or absence of anti-VEGFR2 antibodies. After 30 minutes the inserts were placed into wells containing media with or without

VEGF. Four hours later migration was measured by counting cells that migrated into the wells (Figure 12).



(Excerpted from Zhu et. al.)

Under the conditions of this assay incubation with ramucirumab resulted in dose dependent inhibition of HEL cell migration in response to $VEGF_{165}$.

Report # 1282-02

VEGF Expression in HT-29 Tumor Bearing Mice

This study was performed to quantitate the blood plasma levels of mouse vascular endothelial growth factor (mVEGF) in HT29 human colon adenocarcinoma tumor bearing mice treated with increasing doses of DC101 and to determine whether there is a correlation between mVEGF expression and tumor growth.

Athymic nu/nu mice were injected subcutaneously with 2.0×10^6 HT29 cells in 50% Matrigel™. When the tumors reached an average volume of 200 mm^3 the mice were divided into five groups (n=5) as follows:

- PBS
- DC101 200 µg
- DC101 400 µg
- DC101 800 µg
- DC101 1200 µg

DC101 (as indicated above) was given ip every three days for 4 weeks. Tumors were measured two times per week. Blood samples were collected before treatment was initiated and then once per week during treatment. The mVEGF quantitative determination was performed with the Quantikine® M Mouse VEGF Immunoassay.

HT29 tumor bearing mice treated with DC101 showed a dose dependent increase of mVEGF expression in their blood plasma (Figure 13A). In spite of that, DC101 inhibited HT-29 tumor growth in a dose dependent manner (Figure 13B)

The Applicant reports that in a separate experiment (not submitted), nude mouse blood plasma spiked with increasing amounts of DC101 also increased mVEGF levels in the immunoassay. The Applicant suggests that increased mVEGF expression in these murine plasma samples could be an artifact of the ELISA method, but as increased hypoxia can lead to VEGF induction, this increase could also be evidence of a pharmacodynamic effect of the antibody with higher levels causing deceased vascular support and higher levels of hypoxia. Acute treatment of HT29 tumor bearing nude mice with DC101 may induce increases in mVEGF expression with longer treatment periods leading to inhibition of expression in a time-dependent manner (Figure 13A).

Study Report #1137-02

Efficacy of DC101 on HT-29 (p53 mutant) Xenografts

The ability of DC101 to inhibit the growth of p53 mutant HT-29 colon carcinoma cells was evaluated in athymic nu/nu mice injected sc with $5x10^6$ HT-29 colon carcinoma cells embedded in Matrigel™. When tumors reached 200 mm^3 mice were randomized and divided into two treatment groups (n=10):

- Group 1 was given Rat IgG at 1, and 0.5 mg/day (USP saline after Day 33)
- Group 2 was given DC101 at 1, and 0.5 mg/day

Treatment was given every three days, ip for sixteen weeks and tumor measurements were recorded twice weekly. The control group (Group 1) was terminated after Day 71

due to tumor burden. DC101 therapy significantly inhibited growth of HT-29 xenografts from Day 33 onward compared to controls (Figure 14).

(Excerpted from the Applicant's submission)

In summary, DC101 inhibited growth of implanted HT-29 tumors despite having mutated p53.

Study Report #: 1245-02

DC101 Effects on Tumor Hypoxia in HT-29 Xenografts

In order to evaluate the possible effects of mAb, DC101, on HT-29 tumor cell hypoxia, 5 x 10^6 in Matrigel (1:1) HT-29 tumor cells were injected sc into 10 female athymic nu/nu mice at. Tumors were allowed to reach 150-200 mm^3 and the animals were then divided into two groups (n = 5 each):

- Group 1 was given saline (0.5 mL; q3days for 21 days)
- Group 2.was given DC101 mAb (1 mg/day; q3days for 21 days)

Tumor volume measurements were taken weekly. Tumor cell hypoxia was detected using the Chemicon Hypoxyprobe™-1 kit which consists of a hypoxia marker, pimonidazole hydrochloride, that reportedly binds to oxygen-starved cells, and a mAb, Hypoxyprobe™-1-Mab1, that detects pimonidazole adducts using standard immunohistological methods. 30 min after injection of mAb (1 mg DC101) or saline control in the morning of Day 22 (from start of treatment), Hypoxyprobe Reagent A was

injected into the animals via tail vein. Animals were sacrificed 60 min later and tumors excised for histological processing and Hypoxyprobe staining. At the time of Hypoxyprobe injection on Day 22, the average tumor volume in the control group was 1673 mm^3 compared to 513 mm^3 for the group treated with mAb, DC101, suggesting inhibition of tumor growth by DC101; however, no significant and consistent differences in hypoxyprobe staining were observed between DC101-treated and control animals.

Thus, whereas DC101-treatment reduced tumor volume by an average of more than 50% of control, it had no effect on tumor hypoxia compared to controls.

Study Report #: 1246-02

Efficacy comparison of KDR antibodies IMC-1121, IMC-2C7, IMC-2C6 in HEL leukemia model

Mice were divided into six groups (n=10) and used to compare the anti-leukemic effect of human anti-VEGFR2 antibodies IMC-1121, IMC-2C7 and IMC-2C6 using a human leukemia model, human erythroleukemia cell line (HEL). Each group of mice was pre-treated with 200 rad gamma radiation then two days later, each group of mice was injected intravenously with 5 x 10^6 of tumor cells. Another day later, each group of mice started treatment twice weekly with the doses of antibodies shown below:

A) Human IgG (800 µg, q2w; control group)
B) IMC-1121 (800 µg, q2w)
C) IMC-1121 (200 µg, q2w)
D) IMC-2C7 (800 µg, q2w)
E) IMC-2C7 (200 µg, q2w)
F) IMC-2C6 (800 µg, q2w)

Treatment with 800 µg of IMC-1121, (b) (4) or 200 µg of IMC-1121, (b) (4) significantly prolonged the survival of HEL-injected mice to an average of ≥40 days compared with an average of 31 days in the control group. The treatment groups were not significantly different from each other regardless of the dose or antibody used (Figure 15).

Figure 15: Survival profiles of HEL leukemia mice treated with anti-KDR antibodies 1121, 2C7 or 2C6

(Excerpted from the Applicant's submission)

These results suggest that treatment with anti-VEGFR2 antibodies IMC-1121, (b) (4) prolonged mouse survival in the KDR-positive HEL leukemia model following gamma radiation.

Report # 1304-02

Efficacy of KDR Antibodies in Xenograft HEL Leukemia Model

This study was performed to compare the activity of several anti-VEGFR2 antibodies (IMC-1121, IMC-2C6, or IMC-2C7) in NOD-SCID mice injected with cells from a KDR-expressing human leukemia line, HEL, in order to determine which one should be used for further characterization and potentially for clinical testing.

Mice were injected intravenously with 5 x 10^6 HEL cells per mouse and then divided into 8 groups [(A-H), 10 mice per group] as shown below. One day later, mice began treatment by i.p. injection three times weekly. Mice were monitored daily for survival.

A. Control human Ig (800 µg)
B. 2C6 (800 µg)
C. 2C7 (800 µg)
D. 2C7 (400 µg)
E. 2C7 (100 µg)
F. 1121 (800 µg)
G. 1121 (400 µg)
H. 1121 (100 µg)

Compared to the control (human Ig) group, (b) (4) IMC-1121 treatment (100 µg, 400 µg or 800 µg doses) were not effective (Figure 16).

Figure 16: Efficacy of KDR Antibodies (IMC-1121, IMC-2C6, IMC-2C7) in Xenograft HEL Leukemia Model

(b) (4)

(Excerpted from the Applicant's submission)

(b) (4) . In the absence of gamma radiation, IMC-1121 showed no activity in this model.

Report # 1305-02

Efficacy Study of IMC-1121 in HL-60 Xenograft Leukemia Model

This study was performed to determine 1) the activity of IMC-1121 in the KDR-expressing HL-60 model; 2) the potential additive effect from using the combination of IMC-1121 and DC101, blocking both autocrine and paracrine pathways of VEGF signaling.

Mice were injected iv with $5x10^6$ HL-60 cells per mouse. Mice were then divided into the following groups (10 mice per group):

A. Control human Ig (800 µg)
B. IMC-1121 (800 µg)

C. IMC-1121 (400 µg)
D. IMC-1121 (200 µg)
E. IMC-1121 (800 µg) + DC101 (800 µg)
F. DC101 (800 µg)

One day later, mice began treatment with IMC-1121 (as indicated above) by i.p. injection three times weekly. Mice were monitored daily for survival.

Compared to the control (human Ig) group, IMC-1121 or DC101 treatment (800 µg) significantly prolonged mouse survival in the human promyelocytic leukemia **cell** line (HL-60) leukemia model (

Figure 17) The effect of IMC-1121 treatment (at 400 µg or 200 µg dose) was not significant. Treatment with the combination of IMC-1121 and DC101 showed a significantly enhanced effect on survival compared to either IMC-1121 (800 µg) or DC101 (800 µg) alone (

Figure 17).

Figure 17: Effect of combination of 1121 and DC101 in KDR-expressing HL-60 Xenograft Leukemia model

(Excerpted from the Applicant's submission)

Whereas 800 µg of either IMC-1121 or DC101 alone did not significantly prolong mouse survival in the HL-60 leukemia model, the combination of IMC-1121 and DC101 did. Thus IMC-1121 alone seems to be ineffective in prolonging mouse survival in HEL and HL-60 leukemia models.

Report # 1319-02

Histological Effects of anti-VEGF R2 mAb DC1 01 on p53-mutated HT-29 Xenografts

This study examined the cellular effects of anti-VEGFR2 mAb, DC101, in an HT-29 colon carcinoma Xenograft model. HT-29 has carries a mutated p53 with an Arg-His missense mutation at codon 273.

Athymic *nu/nu* mice were injected sc with 5 x 10^6 cells HT-29 colon carcinoma cells in a volume of 0.4 ml containing 50% Matrigel. When tumors reached ~190 mm^3 mice were randomized into two treatment groups (*n* = 10):

1. Control, USP saline; M-W-F
2. DC101, 1mg/dose; M-W-F

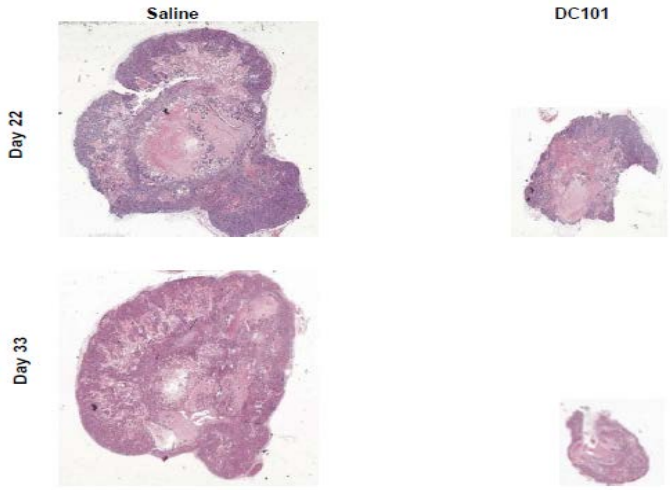
On Days 22 and 33 two animals per group were sacrificed and tumors were resected for histological examination. Fixed tumor sections were examined by H&E staining, staining with an endothelial cell marker, and TUNEL staining. Animals were also sacrificed at Days 8, 15, 22, and 29 for hypoxia evaluation using the Hypoxyprobe kit.

Compared to the control group, DC101 significantly inhibited HT-29 tumor growth, from Days 8 through 29 (Figure 18), with a T/C% value (growth inhibition) of (b) (4) by Day 29. After Day 29, the number of animals left in either group was rather small due to the use of animals at several time points.

(Excerpted from the Applicant's submission)

The decrease in tumor size in the DC101-treated group was confirmed histologically (Figure 19) and an increase in necrotic areas, compared to the control, was observed. Although not quantified, trends towards decreased vascularity, measured by decreased staining using an antibody against the anti-pan endothelial cell antigen MECA-32, and possible mild increases in hypoxia in tumors from DC101-treated animals as measured by Hydroxyprobe staining were observed. The healthy tumor compartment consisted of cells with large nuclei along with some mitotic cells and an adipose-like structure.

Figure 19: H&E Staining of Resected HT-29 Tumors



(Excerpted from the Applicant's submission)

Thus, treatment of HT-29 tumor implanted mice with DC101 significantly inhibited growth of the xenografts and resulted in tendencies towards decreased vascularity, increased hypoxia, and increased apoptosis in tumor samples.

Report # 3493-05

Comparison of the antitumor efficacy of DC 101 and the combination of IMC-18F1 + MF1 in the MDA-MB-231 xenograft model

The objective of this study was to compare the antitumor effects of IMC-18F1 (anti-human VEGFR1) in combination with MF1 (anti-mouse VEGFR1) versus DC101 (anti-mouse VEGFR2) in the MDA-MB-231 breast cancer model.

Treatment of nude mice subcutaneously injected with $6x10^6$ MDA-MB-231cells with DC101 as a monotherapy resulted in decreased tumor growth inhibition compared to the combination of MF1+IMC-18F1. Adding VEGFR1 inhibition to VEGFR2 inhibition

(DC101+IMC-18F1+MF1) resulted in a mild increase in tumor growth inhibition compared to VEGFR1 inhibition alone (Figure 20).

Figure 20: Tumor Growth Curves

(Excerpted from the Applicant's submission)

In summary, the inhibition of VEGFR1, VEGFR2, or both receptors in this breast cancer model, inhibited tumor growth to a similar degree.

Report Number 4719-10

Efficacy of VEGFR mAbs in a Paclitaxel+S12-Refractory SC MDA-MB-231-LP-OS-PT Carcinoma Xenograft Model (IV-1622)

The objective of this study was to determine the effects of anti-VEGFR1 or VEGFR2 mAbs, combined with 5-FU/LV, on the growth of MDA-MB-231-LP-OS-PT breast carcinoma tumors that are resistant to treatment with paclitaxel + anti-VEGF mAb S12.

MDA-MB-231-LP-OS-PT cells (10^7 cells/mouse) were prepared and injected sc into 120 athymic mice. When tumor volumes reached ~300 mm^3 all animals began treatment with one dose of 10 mg/kg paclitaxel (Day -7) and 40 mg/kg anti-VEGF mAb (Days -6, -4, and -2). Paclitaxel and S12 were dosed IP and tumor volumes were measured on the first and eighth day of paclitaxel+S12 therapy.

After the 8th day of paclitaxel+S12 treatment (Day 1), animals with a tumor volume <2x that at the start of treatment ("responders") were removed from the experiment and euthanized while animals with a tumor volume ≥2x the starting volume ("refractory") were randomized by tumor volume to the following treatment groups (*n* = 11-12):

1) USP Saline at 10 µl/g
2) anti-VEGFR2 mAb DC101 at 40mg/kg
3) anti-VEGFR1 mAbs IMC-18F1+MF1, each at 40 mg/kg
4) 5-FU at 125 mg/kg + LV at 62 mg/kg
5) IMC-18F1+MF1 + 5-FU/LV
6) DC101 + 5-FU/LV

5-FU/LV was prepared and administered by ip injection on Days 1, 7, and 14. Monoclonal antibodies were dosed ip, M-W-F, starting one day after the start of 5-FU/LV therapy. Antibodies used in this study were: DC101, IMC-11F8, and MF1. Tumor measurements and body weights were recorded on Days -7, 1, 9, 11, 14 and 17 of mAb treatment. On Day 17, due to high tumor burdens and weight loss, the animals were sacrificed.

At Day 1 of anti-VEGFR mAb +/- 5-FU/LV treatment, tumor volumes in mice selected for paclitaxel+S12 resistance averaged ~740mm^3. Anti-tumor activity was measured by comparing average tumor volumes from treated (T) verses control (C) animals (T/C%).

Figure 21: Growth of MDA-MB-231-LP-OS-PT Xenografts

(Excerpted from the Applicant's submission)

Thus, whereas anti-VEGFR mAb therapy alone had a minimal inhibitory effect on the growth of paclitaxel+S12 resistant MDA-MB-231-LP-OS-PT tumors compared to a saline only control, when either anti-VEGFR2 or VEGFR1 antibodies were combined with 5-FU/LV, significant anti-tumor effects exceeding those of 5-FU/LV alone were observed.

Report Number 3888-07

Comparision of Sutent or DC101 with or without Docetaxel in the MDA-MB-435LM2 Xenograft Model

Treatment of nude mice implanted with $5x10^6$ cells from th human MDA-MB-435LM2 breast cancer line with DC101 (40 mg/kg) had significantly greater antitumor effects than Sutent (40 mg/kg M-W-F), when each compound was given alone or in combination with Docetaxel (5 mg/kg, q7dx3). Combinations of DC101 or Sutent with Docetaxel (12 mg/kg, q7dx3) exhibited antitumor effects that were similar to 12 mg/kg Docetaxel or DC101 monotherapy (Figure 22).

Figure 22: Effects of DC101, Sutent and Docetaxel Treatment on Tumor Volume

(Excerpted from the Applicant's submission)

Report Number 4721-10

VEGFR1-2 +5-FU/LV efficacy in a S12+Paclitaxel-Refractory DU4475 Breast Cancer Xenograft Model (IV-1624)

This experiment was performed to determine the effects of anti-VEGFR1 (IMC-18F1 and MF1) and VEGFR2 (DC101) mAbs, in combination with 5-FU/LV on the growth of DU4475 breast carcinoma tumors that are resistant to S12+paclitaxel therapy.

120 nu/nu mice (female, 7-8 weeks of age) were injected with DU4475 cells at $2.0x10^6$ cells/mouse. When tumor volumes reached ~375 mm^3, all animals were treated with paclitaxel at 10 mg/kg administered q7d, and S12 at 40 mg/kg on a M-W-F schedule. Paclitaxel dosing started on Day -17, 24 hours before the start of S12 dosing. Treatments were administered ip. Tumor volumes were measured twice per week.

On Day 1, the animals with a tumor volume less than or equal to that at the start of treatment ("responders") were removed from the study, and euthanized. The 48 remaining animals ("non-responders") were randomized by tumor volume into the treatment groups (n=12) shown below. No further paclitaxel + S12 treatments were given.

1. USP Saline at 10 µl/g, ip, M-W-F
2. 125/62 mg/kg 5-FU/LV, ip, q7d
3. 40 mg/kg DC101, ip, M-W-F + 125/62 mg/kg 5-FU/LV

4. 40 mg/kg IMC-18F1 + 40 mg/kg MF1 + 125/62 mg/kg 5-FU/LV

Administration of 5-FU/LV started on Day 1, one day before the start of mAbs and saline dosing. Tumor volume measurements and body weights were recorded at least twice per week for the duration of the study. The T/C% was calculated for each treatment group on Day 10 as the ratio of the relative tumor volumes in the experimental versus the control groups.

(b) (4)

(b) (4)

significant improvements on the effect of 5-FU/LV alone, there were trends for increased benefit, particularly with the DC101 combination.

Figure 23: Effect of Treatment on Tumor Volume

(b) (4)

(Excerpted from the Applicant's submission)

The Applicant reports that DU4475 tumors are known to cause significant body weight decrease when growing subcutaneously in nu/nu mice even without treatment, but body weight loss becomes more dramatic when animals with DU4475 tumors are given chemotherapies. In the current study, 4 animals treated with the combination of IMC-18F1+MF1+5-FU/LV with significant body weight loss became moribund on Day 10, therefore, they were euthanized after the Day 10 tumor measurements. Two animals in the 5-FU/LV treatment group and 3 animals in the DC101+5-FU/LV treatment group also died by Day 14.

In conclusion, no significant antitumor benefits, over 5-FU/LV, were observed when 5-FU/LV was combined with DC101 or IMC-18F1+MF1 in this model, although trends for increased benefit, particularly with DC101, were observed.

Report # 5310-12

Anti-VEGFR2 mAb DC101 +/- paclitaxel efficacy in an NCI-N87 human gastric carcinoma Xenograft model (IV-2213)

The objective of this experiment was to determine the efficacy of anti-VEGFR2 mAb DC101 and paclitaxel, alone or in combination, in an NCI-N87 model of gastric carcinoma Xenograft model.

A cell suspension of 3 x 10^6 NCI-N87 cells/mouse was injected sc into the left flank of 60 female athymic mice. When tumors reached ~295 mm^3, the mice were randomized by tumor volume and divided into four treatment groups (*n* = 12):

1) USP saline control, 10 µl/g, M-W-F
2) mAb DC101 at 40 mg/kg, M-W-F
3) Paclitaxel at 20 mg/kg, q7d x 3
4) Combination

Paclitaxel was dosed weekly for three weeks, starting on “Day 0”, one day before the start of mAb DC101 dosing ("Day 1"). USP Saline and DC101 were given ip, M-W-F. T/C% was calculated by the formula 100 x Relative Mean Tumor Volume of the experimental group (Day 32/Day 0) / Relative Mean Tumor Volume of the USP Saline control group.

The average NCI-N87 tumor volume in the USP Saline group was 1169 mm^3 at Day 32. Anti-VEGR2 mAb, DC101, significantly inhibited the growth of NCI-N87 tumors (T/C = (b) (4)) while paclitaxel therapy had no inhibitory effect on the growth of these tumors (Figure 24). The combination of DC101 and paclitaxel significantly inhibited the growth of NCI-N87 tumors compared to USP Saline (T/C = (b) (4)) or paclitaxel alone but the combination did not provide a therapeutic advantage compared to DC101 monotherapy (Figure 24).

Figure 24: Effect of drug combination on the growth of NCI-N87 Xenografts

(Excerpted from the Applicant's submission)

The results of this experiment show that Anti-VEGFR2 mAb DC101 significantly inhibited the growth of NCI-N87 human gastric carcinoma tumors.

Report # 5311-12

Anti-VEGFR2 mAb DC1 01 +/- paclitaxel efficacy in an MKN-45 human gastric carcinoma xenograft model (IV-2214)

The objective of this experiment was to determine the efficacy of anti-VEGFR2 mAb DC101 and paclitaxel, alone or in combination, in an MKN-45 model of gastric carcinoma.

The effects of DC101 and paclitaxel alone and in combination in MKN-45 model of gastric carcinoma were similar to those in NCI-N87 model of gastric carcinoma Xenograft model (Report # 5310-12; Figure 24).

Thus, whereas anti-VEGFR2 mAb DC101 significantly inhibited the growth of MKN-45 human gastric carcinoma tumors, paclitaxel alone had no effect and the combination of mAb DC101 and paclitaxel offered no therapeutic advantage over DC101 alone.

Report Number 4028-07

Anti-PDGF-Rβ mAb 2C5 Combined with VEGFR2 mAb DC101 and Paclitaxel in NCI-H460 NSCLC Xenografts (IV-922)

The objective of this study was to determine whether the addition of anti-PDGFRβ mAb 2C5 could improve the efficacy of VEGFR2 mAb DC101 + paclitaxel in the NCI-H460 NSCLC model.

5 x 10^5 NCI-H460 cells were injected sc. into 45 athymic mice. When tumor volumes reached ~350 mm^3 mice were randomized by tumor volume into three treatment groups (*n* = 12):

1) USP saline, 10 µl/gram
2) mAb DC101 at 40 mg/kg + paclitaxel at 20mg/kg
3) mAbs 2C5 and DC101 (each at 40 mg/kg) + paclitaxel at 20 mg/kg

Antibodies were administered ip M-W-F while paclitaxel was given q7d, for six weeks.

Owing to aggressive tumor growth in this model the animals in the control group were sacrificed at Day 19. The combination of DC101 and paclitaxel significantly inhibited the growth of NCI-H460 tumors (Figure 25), with a T/C o (b) (4) The Applicant reports that historic data with paclitaxel in this model indicates that it does not have any efficacy as monotherapy (T/C% = (b) (4). Though the combination of 2C5 to DC101 and paclitaxel also significantly inhibited the growth of NCI-H460 tumors (Figure 25), the addition of 2C5 resulted in only a modest increase in NCI-H460 tumor growth inhibition compared the effects of DC101 + paclitaxel alone.

Figure 25: Effects of mAbs and paclitaxel on the growth of NCI-H460 Xenografts

(Excerpted from the Applicant's submission)

In summary, the addition of PDGF-Rb mAb 2C5 mAb to DC101 and paclitaxel did not significantly improve the efficacy of DC101 + paclitaxel in the NCI-H460 model of

NSCLC. The Applicant acknowledges that the failure to treat a group of animals with DC101 alone makes it difficult to know the effect of paclitaxel or 2C5+paclitaxel on DC101 in this model.

In a separate experiment (Report # 3820-06), antibody combination therapy with 2C5 and DC101 significantly inhibited the growth of NCI-H460 tumors compared to 2C5 or DC101 monotherapy in the NCI-H460 model (Figure 26).

(Excerpted from the Applicant's submission)

In yet another experiment (Report # 3730-06) administration of Anti-PDGFRβ mAb 2C5 (40 mg/kg, ip) did not inhibit the growth of HCT-8 human colon tumors and its combination with DC101 (40 mg/kg) did not offer any therapeutic advantage over treatment with DC101 alone.

In a study in HCT-116 colorectal cancer model (Study Report #3821-06), administration of the Anti-PDGFRβ mAb 2C5 (40 mg/kg, ip) in combination with DC101 significantly enhanced the inhibitory effect of DC101 alone in HCT-116 colorectal tumors.

Report # 3608-06

VEGFR1, VEGFR2 Combined Inhibition in the GEO Colon Cancer Xenograft Model

The objective of this study was to determine whether, DC101 (anti-mouse VEGFR2) + 18FI (anti-human VEGFR1), DC101 + MF1 (anti-mouse VEGFR1) or the combination of

all three antibodies, is most effective in the GEO human colon carcinoma xenograft model.

Administration of DC101 (40 mg/kg) combined with MF1 (40 mg/kg) or 18F1 (40 mg/kg) ip M-W-F into female nu/nu mice significantly inhibited GEO tumor growth through day 29 with T/C% values of (b) (4) respectively. There was no statistical difference between these pairwise treatment groups. Combining all three antibodies did not significantly improve the inhibitory effects of either DC101 monotherapy or both pairwise combination groups.

In a separate experiment (Report # 3648-06), treatment of mice implanted with GEO cells with DC101 (40 mg/kg ip M-W-F) was shown to result in increased levels of tumor active HIF-1 and human VEGF as well as reduced tumor blood vessel density. IMC-A12 (an anti-IGFR antibody) +cetuximab, or cetuximab alone, prevented the increase in active HIF-1 and human VEGF induced by DC101, when given in combination. In addition, cetuximab+DC101 also caused prolonged reduction in AKT phosphorylation.

Project # LLI-FTE-C0116201 O.S2.8

In Vivo Assessment of LLY-31 C1, DC101 and S12 as Single Agents in the Treatment of Subcutaneous GAF087 Gastric HuPrime Xenograft Model

The objective of this study was to evaluate the *in vivo* therapeutic efficacy and target inhibition of LLY-31C1 (rat antibody against mouse VEGFR-3), DC101, and S12 (human antibody that targets both human and mouse VEGF-A) as single agents in the treatment of athymic (Balb/c nude) mice subcutaneously implanted with primary human gastric tumor fragments (GAF087).

Human primary gastric tumor fragments (GAF087) reaching the size of 500-700 mm^3 were harvested and equally cut into small fragments of 2-4 mm in diameter. Each mouse was inoculated subcutaneously at right front flank with one tumor fragment. The animals were randomized into 4 treatment groups [as shown below (n = 8)] when the mean tumor size reached approximately 167 mm^3. Doses of antibodies indicated were administered 3x/week for 21 days. Tumor size was measured twice weekly.

1. Hu IgG 40 mg/kg , ip, 3x/week
2. LLY-31C1 40 mg/kg, ip, 3x/week
3. DC101 40 mg/kg, ip, 3x/week
4. S12 40 mg/kg, ip, 3x/week

The mean tumor size of the control (HulgG treated) group reached 1,076 mm^3 at Day 41 after tumor inoculation. Treatment with DC101 at dose level of 40 mg/kg resulted in significant antitumor activity (Figure 27); the mean tumor size being 185 mm^3 at Day 41 (T/C value = (b) (4).

DC101 was well tolerated and animals in this group showed no significant weight loss compared to the control group.

(Excerpted from the Applicant's submission)

In summary, at the 40 mg/kg dose level (Group 3) DC101 showed significant antitumor activity in GAF087 gastric HuPrime Xenograft model without adversely affecting the body weight.

Report # 5538-12

In Vivo Evaluation of DC101 as a Single Agent in the Treatment of Subcutaneous Gastric HuPrime Xenograft Models

Similar experiments to that described above in Project # LLI-FTE-C0116201 O.S2.8 were conducted to evaluate the efficacy of LLY-31C1 (rat antibody against mouse VEGFR-3), DC101 (rat antibody against murine VEGFR2) or S12 (human antibody that targets both human and mouse VEGF-A) as single agents in 16 other subcutaneous primary human gastric models (HuPrime) Xenograft models (Table 3).

Subcutaneous HuPrime models were established as follows: small (2-4 mm in diameter) fresh tumor fragments were prepared from a variety of human primary gastric tumors. BALBC/nude mice (6-8 weeks of age) were subcutaneously implanted with one tumor fragment in the right front flank. When tumors reached an average volume of ~150 mm^3 (range 132-193 mm^3), the animals were randomized by tumor volume into treatment groups as indicated below (n=7-8):

1. Hu IgG 40 mg/kg , ip, 3x/week
2. LLY-31C1 40 mg/kg, ip, 3x/week

3. DC101 40 mg/kg, ip, 3x/week
4. S12 40 mg/kg, ip, 3x/week

Treatments were administered intraperitoneally (ip) at 40 mg/kg, on a three times per week schedule for 3 weeks ending on Study Day 21 (LLY-31C1 was not tested on GAM044 and GAM046 HuPrime models). Tumor size and body weights were measured twice per week. The percent treatment/control (T/C) values were calculated using the following formula: (b) (4), where T = mean tumor volume of the drug-treated group on the final day of the study, and C = mean tumor volume of the control group on the final day of the study.

All three mAbs were generally well-tolerated in HuPrime tumor-bearing mice as determined by body weight. The antitumor effect of DC101 ranged from a T/C of (b) (4) in the GAF087 model, to no antitumor effect corresponding to a T/C of (b) (4) in the GAF023 model; (Table 3). In 10 of 17 models DC101 resulted in significant tumor growth inhibition (TGI) of (b) (4) or greater compared to the vehicle control.

Table 3: Effects of mAbs on tumor volume in a variety of HuPrime models

Report No.	HuPrime Model	DC101		S12		LLY-31C1	
		%T/C	p	%T/C	p	%T/C	p
LLI-FTE-C01162010.S2.3	GAM098	(b) (4)					
LLI-FTE-C01162010.S2.5	GAM037						
LLI-FTE-C01162010.S2.6	GAM019						
LLI-FTE-C01162010.S2.7	GAM016						
LLI-FTE-C01162010.S2.8	GAF087						
LLI-FTE-C01162010.S2.9	GAM044						
LLI-FTE-C01162010.S2.10	GAM046						
LLI-FTE-C01162010.S2.12	GAF055						
LLI-FTE-C01162010.S2.13	GAM110						
LLI-FTE-C01162010.S2.14	GAM060						
LLI-FTE-C01162010.S2.15	GAM119						
LLI-FTE-C01162010.S2.16	GAF023						
LLI-FTE-C01162010.S2.17	GAF114						
LLI-FTE-C01162010.S2.18	GAM022						
LLI-FTE-C01162010.S2.19	GAM025						
LLI-FTE-C01162010.S2.21	GAM0139						
LLI-FTE-C01162010.S2.23	GAM093						

(Excerpted from the Applicant's submission)

In conclusion, DC101 showed an antitumor effect corresponding to TGI value of (b) (4) or greater in 10 of 17 HuPrime models, following administration at 40 mg/kg, ip, three times per week.

Report Number 4087-07

DC101 +/- Sorafenib Efficacy In a SK-Hep1 Xenograft Model

The objective of this study was to evaluate the antitumor effects of DC101 and sorafenib, a small molecule VEGFR2 inhibitor, alone or in combination, in the SK-Hep1 hepatocellular carcinoma xenograft model.

Nu/nu female mice (7-8 weeks of age) were injected sc with $10X10^6$ SK-Hep1 cells/mouse. When tumors reached approximately 300 mm^3, mice were randomized by tumor size into the following treatment groups (n=12):

1. USP saline 10 µl/gram, ip, 3x/week
2. 40 mg/kg DC101, ip, 3x/week
3. 100 mg/kg sorafenib, po, daily
4. 40 mg/kg DC101, ip (3x/week) + 100 mg/kg sorafenib, po (daily)
5. 40 mg/kg Rat IgG, ip, 3x/week

One animal in the sorafenib group was found dead on Day 13, 2 more animals were found dead on Day 18, and all animals (100%) in this group were dead by Day 23. Therefore, results were analyzed up to Day 13. Sorafenib-treated animals also developed skin rash. There were no mortalities in the DC101+sorafenib group.

Treatment with either DC101 or sorafenib (through Day 13) monotherapy significantly inhibited the growth of SK-Hep1 tumors (Figure 28), with T/C%s of (b) (4) respectively. Co-administration of DC101 and sorafenib significantly improved the antitumor effects observed compared to either DC101 or sorafenib alone.

(Excerpted from the Applicant's submission)

In summary, significant antitumor benefits were observed when DC101 was added to sorafenib treatment in this model

Report # 4597-09

HuH-7 #6MPJ Efficacy of DC101 in a Sorefenib-Refractory SC HuH-7 Hepatic Carcinoma Xenograft Model

Effects of DC101 were studied in sorafenib-refractory HuH-7 hepatic tumors. Anti-VEGFR mAb DC101, given at a dose of 40 mg/kg, M-W-F, significantly inhibited the growth of sorafenib-refractory HuH-7 xenografts implanted in athymic mice. Mice with tumors that had grown to 100% compared to control treated cells over 8 days of sorafenib treatment were re-randomized to receive either DC101 or a rat IgG control antibody. Tumors of mice treated with DC101 grew significantly less than those of control treated animals (T/C= (b) (4)).

4.2 Secondary Pharmacology

No studies submitted

4.3 Safety Pharmacology

The Applicant did not conduct stand-alone safety pharmacology studies. Effects of ramucirumab on blood pressure and ECG were assessed as part of toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

No studies submitted except in incisional wound healing studies (see Section 10: Special Toxicology Studies)

6.2 Repeat-Dose Toxicity

Study title: A Five-Week Repeat-Dose Intravenous Toxicity Study of IMC-1121B in Cynomolgus Monkeys with a Terminal Sacrifice and Six-Week Recovery Phase

Study no.:	CRO950
Study report location:	eCDT: 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 28, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	IMC-1121B; Lot # 1278-87and Purity, 98%

Key Study Findings

- Minimal to moderate focal degeneration of skeletal muscle and mononuclear cell infiltration
- Severe thymic atrophy at 40 mg/kg
- Increased CK level at all doses in males and HD female on Day 32

Methods

Doses:	0, 4, 12, 40 mg/kg
Frequency of dosing:	Days 1, 15, 22, and 29 (once a week)
Route of administration:	IV infusion
Dose volume:	4.3 mL/kg for control and high dose groups; 0.4 mL/kg for low dose group and 1.3 mL/kg
Formulation/Vehicle:	Clear colorless liquid/Phosphate buffered saline
Species/Strain:	Cynomolgus Monkey
Number/Sex/Group:	6 males and 3 females/low, mid and high doses, 2 males and 4 females in the control group.
Age:	Not provided
Weight:	Males, 2.13-5.26 kg; females, 1.92-3.28 kg
Deviation from study protocol:	On Day 15, the Experimenter found that animal #6 (in the control group) was a female, erroneously classified as a male. Animal #6 was added to the females thus, the control group ended up with 2 male and 4 females.

Observations and Results

There were no drug-related changes in clinical signs, body weight, food consumption, ophthalmoscopic observations, ECG or urinalysis.

Hematology

Changes in hematologic parameters were neither time- nor dose-related as seen in Table 4 below.

Table 4: Percentage change in hematologic parameters from control values

Sign	Sex	0				4 mg/kg				12 mg/kg				40 mg/kg			
Study Day→		-1	15	24	32	-1	15	24	32	-1	15	24	32	-1	15	24	32
Leucocyte $x10^3/\mu L$	M	9.99	9.28	7.72	10.23	↑20	↑7	↑15	↓3	↑16	↑44	↑51	↑29	↑20	↑32	↑17	↑55
	F	11.98	11.35	9.22	10.87	↓27	↓32	↓8	↓5	↓22	↓21	↓23	↓3	↓22	--	↑10	↑6
Lymphocytes $x10^3/\mu L$	M	4.99	3.42	3.79	3.82	↑8	↑11	↑14	↑18	--	↑63	↑73	↑78	--	↑20	↑33	↑68
	F	5.39	6.02	5.54	6.24	--	↓36	↓29	--	↓19	↓34	↓19	--	↓13	↓23	--	↓9
Reticulocytes %	M	0.3	0.8	0.6	0.9	--	↑25	↑12	↑52	↑27	↑78	↑45	↑103	↑20	↑38	↑28	↑78
	F	0.38	1.55	0.75	1.5	↓20	--	↓20	↑11	↓20	↑37	↑73	↑47	↓28	--	↑16	↑49

Clinical Chemistry

Changes in clinical chemistry parameters were neither time- nor dose-related as seen in Table 5 below. An increase in CK of ≥ 200% was observed in male animals at all doses, and females at the 40 mg/kg dose level.

Table 5: Percentage change in clinical chemistry parameters from control values

Parameter	Sex	0				4 mg/kg				12 mg/kg				40 mg/kg			
Study Day→		-1	15	24	32	-1	15	24	32	-1	15	24	32	-1	15	24	32
ALT[1] U/L	M	29.5	38.5	35	33.5	↑42	↑57	↑29	↑48	↑39	↑162	↑118	↑92	↑85	↑129	↑58	↑41
	F	38.3	37.8	44.5	32.5	↓17	↑131	↑31	↑63	↑44	↑92	↑19	↑31	↑94	↑191	↑69	↑113
Total Bilirubin mg/dL	M	0.5	0.5	0.8	2.15	↑16	↑54	↑28	↓75	↑10	↑66	↓12	↓80	--	--	↓16	↓77
	F	0.6	0.7	1.2	0.45	↑38	↑18	↑6	↓27	↓22	--	↓50	↓27	↓5	↓24	↓22	↑33

Parameter	Sex	0				4 mg/kg				12 mg/kg				40 mg/kg			
Study Day→		-1	15	24	32	-1	15	24	32	-1	15	24	32	-1	15	24	32
Total cholesterol mg/dL	M	135	139	135	146	--	↑12	↑17	↓6	↑9	↑38	↑40	↑18	↑6	↑21	↑16	↓10
	F	134	144	152	131	--	↑5	--	↑5	↑18	↑23	↑24	↑28	↑8	↑22	↑10	↑19
CK[2] U/L	M	85.5	68.5	203	140	↑54	↑225	↑74	↑357	↑13	↑38	↑35	↑245	↑28	↑29	↑19	↑206
	F	80.3	90	150	117	↑25	↑52	↑30	↑164	↑28	--	↑26	↑34	↑4	--	↑131	↑327
Creatinine mg/dL	M	0.9	0.75	0.75	0.75	--	--	--	--	--	↑17	↑29	--	--	↑33	↑27	↑16
	F	0.65	0.55	0.6	0.58	--	↑9	--	--	↑12	↑27	↑28	↑16	↑28	↑58	↑28	↑16
Phosphorus[3] mg/dL	M	5.7	3.8	4.2	4.2	--	↑20	↑25	--	↑7	↑51	↑50	↑14	↓4	↑30	↑45	--
	F	5.8	4.73	5.48	3.88	↓12	↑3	↓10	↓7	↓14	↑7	↑13	--	↓16	↓6	↑3	↑5
Triglycerides mg/dL	M	27	19	19.5	19	↑54	↑129	↑85	↑79	↑53	↑136	↑118	↑141	↑31	↑126	↑96	↑91
	F	49.8	48.8	39.3	48.5	--	↓16	↑22	↓29	↓25	↓25	↑4	↑7	↑5	↓5	↓23	↓16
Potassium mEq/L	M	5.1	4.45	4.95	6	↑3	↑7	↑7	↓24	↓4	↑19	↑12	↓24	↑4	↑21	↑7	↓24
	F	5	4.58	5.25	4.3	↑7	↑22	↑7	--	↓6	↑7	↓10	--	↓6	↑5	--	↑9

-1 – Predose; ↑ - increase; ↓- decrease; -- - no change

[1]Alanine Aminotransferase

[2]CK – Creatine phosphokinase

[3]Inorganic phosphorus

Gross Pathology

One male animal (#30) at the 12 mg/kg dose group was reported to have small epididymides, small prostate, small seminal vesicles and small testes. Also, animal (#36) in the 40 mg/kg group had small seminal vesicles. These observations may not be treatment related as they were not dose related, were seen in only one animal/dose group, and there were no corresponding histopathological findings.

Organ Weights

There were no significant changes in organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

The majority of histopathologic findings consisted of minimal or mild mononuclear infiltration in a variety of organs (Table 6). Minimal to moderate focal muscle fiber degeneration and mononuclear cell infiltration in skeletal muscle was observed only in treated animals.
Moderate and severe thymic atrophy were observed at 4 mg/kg and 40 mg/kg dose levels, respectively.

Table 6: Incidence of histopathologic findings

Microscopic Findings		0		4 mg/kg		12 mg/kg		40 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
Brain stem									
Mononuclear cell infiltration, perivascular	Minimal	--	1	--	--	--	--	1	1
Cerebrum (parietal lobe)									
Mononuclear cell infiltration, perivascular	Minimal	--	1	--	--	--	1	1	1
Colon									
Brown pigment, submucosa	Mild	--	--	--	--	1	--	--	--
Mineralized granuloma, muscular wall	Mild	--	--	1	--	--	--	--	--
Eye ball/Optic nerve (Right)									
Mononuclear cell infiltration, choroid	Minimal	--	--	--	--	--	--	1	--

Microscopic Findings		0		4 mg/kg		12 mg/kg		40 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
Femur with knee joint (Left)									
Decreased width, growth plate	Minimal	--	--	1	--	--	--	--	--
	Mild	--	--	--	--	1	--	--	--
	Moderate	--	--	--	--	--	--	1	--
	Severe	--	--	--	1	--	--	--	--
Femur with knee joint (Right)									
Decreased width, growth plate	Mild	--	1	--	--	1	1	--	--
	Moderate	--	--	--		--	--	1	--
	Severe	--	--	--	1	--	--	--	--
Heart (Left ventricle)									
Mononuclear cell infiltration, interstitium	Minimal	--	--	--	--	1	--	1	--
Mononuclear cell infiltration, subendocardial	Minimal	--	--	--	--	1	2	--	--
Injection site, cephalic vein									
Mononuclear cell/polymorphonuclear cell infiltration, perivascular	Minimal	--	--	--	--	--	--	2	--
Injection site, saphenous vein									
Mononuclear cell/polymorphonuclear cell infiltration, perivascular	Mild	--	--	1	--	1	--	2	--
Kidney (Left)									
Mononuclear cell infiltration, pelvis, submucosa	Mild	--	--	1	--	--	--	--	--
Kidney (Right)									
Mononuclear cell infiltration, interstitium/perivascular	Minimal	--	--	1	--	1	--	2	--
Liver									
Mononuclear cell infiltration, perivascular/sinusoidal	Minimal	1	1	3	--	3	1	1	2
Lungs									
Granuloma with eosinophils perivascular	Mild	--	--	--	--	--	--	--	1
Granuloma, multifocal	Mild	--	--	--	--	--	1		
LN (Mandibular, left)									
Eosinophil increase, medulla	Minimal	--	--	--	--		--	1	--
	Mild	--	--	--	--	1	--		--
Extramedullary hematopoiesis	Minimal	--	--	--	--		--	1	--
	Mild	--	--	--	--	1	--		--
Hyperplasia, lymphoid follicles	Mild	--	--	--	--	1	--	1	--
Parathyroids									
Mononuclear cell infiltration	Minimal	--	--	--	1	1	--	1	--
	Mild	--	--	--	--	--	1	--	--
Seminal Vesicle									
Mineralization	Minimal	--	--	--	--	--	--	1	--

Microscopic Findings		0		4 mg/kg		12 mg/kg		40 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
	Mild	--	--	--	--	2	--	--	--
Skeletal muscle (quadriceps, femoris, left)									
Mononuclear cell infiltration, focal	Minimal	--	--	--	1	--	--	1	1
Muscle cell degeneration and mononuclear cell infiltration, focal	Minimal	--	--			1	--	--	--
	Mild	--	--	1	1		--	1	--
	Moderate	--	--	--	--	--	--	1	--
Sacrocysts	Minimal	--	1	1	1	--	--		1
	Mild	--	--	--	1	1	--	1	1
Stomach									
Mononuclear cell/eosinophil infiltration, submucosa, focal	Moderate	--	--	--	--	--	--	1	--
Thymus									
Atrophy	Moderate			1					--
	Severe	--	--	--	--	--	--	1	--
Thyroids		--	--	--	--	--	--		--
Ectopic thymus	Present	--	1	2	--	--	--	1	2
	Minimal	--			--	--	--	1	--
Mononuclear cell infiltration, interstitium	Mild	--	--	1	--	1	1	--	--

-- - no change

Toxicokinetics

As shown in Table 7, the mean AUC increased in a greater than dose proportional manner for the 4, 12 and 40 mg/kg dose groups. The mean C_{max} behaved in a similar manner for the 4, 12 and 40 mg/kg dose groups and the half-life of ramucirumab increased with increasing dose.

Table 7: Toxicokinetic Parameters

	4 mg/kg	12 mg/kg	40 mg/kg
C_{max} (µg/mL)	122	393	1460
AUC_{inf} (h*µg/mL)	7572	35868	189150
$T_{1/2}$ (h)	54.6	97.5	143.1

In immunogenicity testing, 7 of 8 animals in the 4 mg/kg dose group, 2 of 7 in the 12 mg/kg, and 2 of 7 in the 40 mg/kg dose groups were positive for anti-ramucirumab antibodies; however, adequate drug exposure appeared to be maintained.

Study title: Thirty-Nine Week Toxicity Study of IMC-1121B in Cynomolgus Monkeys

Study no.:	1163-110
Study report location:	eCTD: 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 04, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	IMC-1121B; Lot # 1278-91/04A00161; Purity 100%

Key Study Findings

- Minimal chronic inflammation in the kidney and liver of females sacrificed on Day 85
- Moderate to severe glomerulonephritis at MD and HD in terminally sacrificed male and female animals
- Inflammation of various sections of the GI tract
- Thickening of the epiphyseal growth plates at doses ≥ 16 mg/kg and osteochondrapathy (abnormal ossification with cartilage cell retention) at all dose levels

Methods

Doses:	0, 5, 16, 50 mg/kg
Frequency of dosing:	Once every week
Route of administration:	IV infusion
Dose volume:	Control and HD groups, 5.05 mL/kg; LD group 0.52 mL/kg; MD dose group,1.62 mL/kg
Formulation/Vehicle:	Phosphate Buffered Saline
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group for 39-week study; 3 females/group for 12-week study; no recovery groups
Age:	Young adults (>3 years old)
Weight:	Males, 2.5-3.1 kg; Females, 1.9-2.3 kg
Unique study design:	3 females/dose group were sacrificed after 12 weeks of treatment

Observations and Results

Mortality

One female animal (#10140) in the 5 mg/kg dose group was found dead on Study Day 5 following a routine teeth blunting procedure. No gross pathology findings were noted, and microscopic findings were typical of acute interstitial pneumonia, to which the

sedative/anesthesia used during the teeth-blunting procedure may have contributed. All other study animals survived until their protocol-specified scheduled termination.

Clinical Signs

Common clinical observations included abrasions, alopecia, loss of appetite, dehydration, diarrhea, discolored feces, dyspnea, edema, emesis, erythema, mucoid feces, nasal discharge, soft feces, thinness, and ungroomed and matted fur. These observations occurred in all groups (treatment and control) with no relationship to dose level time and were not considered test article-related.

Body Weights

There were no consistent changes in body weight; however, the body weight of male animal #10151 dosed at 50 mg/kg increased by 32% by week 39, relative to the starting weight (4.1 kg) compared to a change of less than 20% in the other two monkeys.

Feed Consumption

There were no remarkable changes in feed consumption.

Ophthalmoscopy

No remarkable changes in ophthalmic findings

ECG

Electrocardiograms were within normal limits.

Hematology

Changes in hematologic parameters were inconsistent and difficult to interpret in the light of changes in baseline values.

Table 8: Percentage change in hematologic parameters from control values

Sign	Sex	0				5 mg/kg				16 mg/kg				50 mg/kg			
Study Day→		-1	29	85	273	-1	29	85	273	-1	29	85	273	-1	29	85	273
WBC $x10^3/\mu L$	M	13.24	11.13	10.46	5.53	--	↑48	↑48	↑82	↓19	↑37	↑42	↑97	--	↑11	↑24	↑121
	F	11.84	11.41	12.55	8.37	--	--	--	↑6	--	↑25	↑15	↑56	--	--	↑12	↑40
Platelets $x10^3/\mu L$	M	277.3	409.7	390.7	342.3	↑50	↑25	↑52	↑30	↑43	↑4	↑32	↑7	↑48	↑25	↑32	↑5
	F	394.3	392.2	492.8	314.3	↑18	↑26	↑6	↑28	↑10	↑25	↑15	--	↑25	↑26	↑9	--
Neutrophils $x10^3/\mu L$	M	8.94	4.99	4.02	1.32	↓6	↑79	↑88	↑181	↓41	↑17	↑36	↑120	↓15	--	↑17	↑246
	F	6.14	5.03	5.43	3.16	--	↓15	↓10	↓19	--	↓12	--	↑38	--	↓17	↓17	↑35
Lymphocytes $x10^3/\mu L$	M	3.78	5.58	5.56	3.90	↑21	--	↑16	↑48	↑28	--	↑41	↑86	--	--	↑27	↑72
	F	4.74	5.53	5.82	4.73	↓12	↑20	↑17	↑21	--	↑57	↑27	↑65	--	--	↓8	↑41
Monocytes $x10^3/\mu L$	M	0.21	0.21	0.49	0.19	↑48	↑110	↑43	↑32	--	↑152	↑59	↑74	--	↑90	↑27	↑121
	F	0.39	0.39	0.58	0.22	--	--	↓12	↑32	--	↑36	↑33	↑82	↓28	--	↓19	↑91
Eosinophils $x10^3/\mu L$	M	0.30	0.33	0.39	0.11	--	↑70	↑90	↑200	--	↑103	↑95	↑245	↑67	↑36	↑38	↑355
	F	0.56	0.45	0.70	0.25	↓21	--	↓37	--	↓21	↑33	↑13	↑88	↓37	--	↓28	↑40

-1 – Predose; ↑ - increase; ↓- decrease; -- - no change

Table 9: Change in activated partial thrombin time (seconds) from control values

	Sex	0				5 mg/kg				16 mg/kg				50 mg/kg			
		-1	29	85	273	-1	29	85	273	-1	29	85	273	-1	29	85	273
APTT (sec)	M	25.35	40.70	28.94	23.96	↑5.6	↓8.0	--	--	--	↓14.0	↓6.1	↓4.2	--	↓9.8	↓6.4	↓3.8
	F	28.63	32.64	26.93	20.02	↓4.1	↓4.6	↓2.1	--	↓6.3	↓11.0	↓4.8	↓1.7	--	↓5.8	↓1.4	--

-1 – Predose; ↑ - increase; ↓- decrease; -- - no change

16.7 seconds for Males; 12.6 second in female control animals

Clinical Chemistry

Changes in clinical chemistry parameters were neither time- nor dose-related, as seen in Table 10.

Table 10: Percentage Change in clinical chemistry parameters from control (0) values

Sign	Sex	0				5 mg/kg				16 mg/kg				50 mg/kg			
Study Day→		-1	29	85	273	-1	29	85	273	-1	29	85	273	-1	29	85	273
Glucose mg/dL	M	44.33	70.33	76.67	48.67	↓18	↓28	--	--	↑32	↓12	↑13	↑16	--	↓25	--	--
	F	43.50	52.50	73.83	53.0	↑26	↑9	↑19	↓25	↑15	↑19	--	↓15	↑36	↑23	↑28	--
BUN mg/dL	M	21.67	20.67	18.0	20.67	--	--	↑17	--	--	↑6	--	↑13	--	--	--	↑66
	F	20.33	20.17	19.33	20.67	--	--	↓16	--	--	--	--	↑48	↑16	--	--	↑24
Cholesterol mg/dL	M	144.3	163.3	180.3	163.0	--	↓9	--	↓14	↑14	↑10	↓20	↑31	↑11	↑9	↓18	↑90
	F	152.2	163.8	175.0	160.0	↑29	↑11	↑8	--	↑5	↑5	--	↑81	--	↑10	--	↑17
Triglycerides mg/dL	M	40.67	70.33	53.33	41.33	↑39	15	↓18	--	31	↑9	↑23	↑89	↑34	↓11	↑49	↑153
	F	53.0	73.8	60.50	45.0	↑84	↓12	↓13	↓13	↓10	↓14	--	↑77	--	↓5	↓9	↑41
AST U/L	M	77.33	57.33	67.0	75.33	--	--	--	↑7	↓24	--	↑16	↑7	--	--	--	↑41
	F	53.33	50.50	47.50	55.67	--	--	↑10	↑34	↑13	--	↑29	↑44	↑26	--	↑47	↑29
Bilirubin mg/dL	M	0.37	0.47	0.33	0.37	--	↓43	↓30	↑8	--	↓30	↑21	↑16	↑27	↓36	↓39	↑16
	F	0.42	0.48	0.37	0.53	↓29	↓31	↓24	↓25	↓33	↓27	↓24	↓30	↓10	↓27	--	↓38
Creatine Kinase U/L	M	607.3	268.0	587.3	362.3	↓37	↓41	↑25	↑58	↓70	↓25	↑31	↑23	↓38	↓39	↓34	↑788
	F	205.8	904.8	164.8	268.7	↓10	↓76	↑8	↑56	↓29	↓53	↑141	↑191	↓227	↓86	↑310	↑269

-1 – Predose; ↑ - increase; ↓- decrease; -- - no change red: large variability among control animal values

Urinalysis

No significant findings

Gross Pathology

One male monkey (#10151), treated at 50 mg/kg dose level, was observed with a swollen scrotum and lower extremities from SD 212 until termination on SD 274. This animal had a high blood urea nitrogen and cholesterol concentration, high creatinine, low albumin concentration, and proteinuria reported in clinical pathology evaluations on SD 183 and SD 273. In addition, there was a histopathologic finding of renal damage. Therefore, the swollen scrotum and lower extremities could be attributed to protein loss, secondary to the ramucirumab-induced renal damage.

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Histological Findings in female animals sacrificed on SD 85

In addition to minimal mononuclear cell infiltration in various organs, minimal chronic inflammation was observed in the kidneys at all doses and liver at high dose (Table 11).

Table 11: Histopathologic findings in animals sacrificed on Study Day 85

Microscopic Findings		0	5 mg/kg	16 mg/kg	50 mg/kg
		F	F	F	F
	Group Size:	3	3	3	3
	Grade	Study Day 85 sacrifice)			
Kidneys					
Inflammation; chronic; Interstitial; focal	Minimal	--	1	1	2
	Mild	--	--	1	--
Ectasia; tubular; multifocal	Minimal	--	--	--	1
Lacrimal glands					
Infiltrate(s); lymphocytic; periductal; focal	Mild	--	1	1	1
Liver					
Infiltrate(s); mononuclear-cell; periportal; multifocal	Minimal	--	1	1	1
Inflammation; chronic; multifocal	Minimal	--	--	--	2
Hyperplasia; multifocal	Minimal	--	--	1	--
Lungs					
Infiltration; lymphoid; multifocal	Minimal	1	1	3	2
Pigmentation; multifocal	Present	--	1	--	2
Pancreas					
Infiltrate(s); lymphoid; periductal	Mild	--	--	1	--

Microscopic Findings		0	5 mg/kg	16 mg/kg	50 mg/kg
		F	F	F	F
	Group Size:	3	3	3	3
	Grade	Study Day 85 sacrifice)			
Mand. salivary glands					
Infiltrate(s); lymphoid; multifocal	Minimal	--	1	2	1
Trachea					
Infiltrate(s); lymphocytic; submucosal; difuse	Minimal	--	--	--	1
Inflammation; granulomatous; submucosal; focal	Mild	--	1	--	1
Thyroid glands					
Infiltrate(s); lymphoid; focal	Minimal	--	--	1	--

Histological Findings in animals sacrificed on SD 274:

Kidney toxicity was evident as moderate and severe glomerulonephritis at 16 and 50 mg/kg dose levels, in male and female animals. Minimal to moderate inflammation of some sections of the GI tract was observed, liver, and skin were observed in the 50 mg/kg group animals. Mineralization or inflammation of some parts of the brain, ovaries and the thymus, mainly in female animals, were observed at the 16 and 50 mg/kg dose levels but the severity was not graded. Reported severe hemorrhage in the skin on one high dose animal seems to be away from the injection site.

Table 12: Histopathologic findings in animals sacrificed on Study Day 274

Microscopic Findings		0		5 mg/kg		16 mg/kg		50 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
Adrenals									
Vacuolation; eosinophilic; zona fasciculata; unilateral; focal	Minimal	--	--	--	--	--	--	1	--
Brain									
Mineralization; cerebral; grey matter; focal	Present	--	--	--	--	1	--	--	2
Inflammation; lymphoplasmacytic; vascular; grey matter; focal	Present	--	--	--	--	--	--	--	1
Cuffing; lymphocytic; grey matter; focal	Present	--	--	--	--	1	--	1	--
Cuffing; lymphocytic; meningeal; focal	Present	--	--	1	--	1	--	--	--
Cuffing; lymphocytic; choroid plexus; focal	Present	--	--	1	--	1	--	1	--
Colon									
Inflammation; subacute; diffuse	Moderate	--	--	--	--	--	--	1	--

Microscopic Findings		0		5 mg/kg		16 mg/kg		50 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
Epididymides									
Edema; bilateral; interstitial; diffuse;	Moderate	--	--	--	--	--	--	1	--
Esophagus									
Infiltration; lymphoid; submucosal; multifocal	Minimal	--	--	1	--	--	1	2	1
Heart									
Aggregates; mononuclear-cell; multifocal	Minimal	--	--	3	2	1	1	1	--
Injection site									
Perivasculitis; lymphoplasmacytic; right; focal	Minimal	--	--	--	--	--	--	2	--
Kidneys									
Glomerulonephritis; diffuse	Moderate	--	--	--	--	1	2	1	2
	Severe	--	--	--	--	1	1	1	1
Glomerulonephritis; multifocal	Moderate	--	--	--	--	1	--	1	--
Lacrimal glands									
Infiltrate(s); lymphocytic; periductal; multifocal	Minimal	--	--	--	--	1	1	1	1
Liver									
Inflammation; caseous; multifocal	Mild	--	--	--	--	--	1	--	--
Vacuolation; pericentral; Multifocal	Mild	--	--	--	--	--	--	1	--
Lungs									
Foamy macrophages; multifocal	Minimal	1	1	1	1	1	1	3	2
Ovaries									
Mineralization; follicular; multifocal	Present	--	1	--	1	--	2	--	3
Pancreas									
Infiltrate(s); lymphoid; focal	Minimal	--	--	--	1	--	--	1	--
Skin									
Inflammation; fibrino-purulent; subcutaneous; multifocal	Moderate	--	--	--	--	--	--	1	--
Hemorrhage; subcutaneous; diffuse	Severe	--	--	--	--	--	--	1	--
Vasculitis/Perivasculaitis; fibrino-purulent; multifocal	Moderate	--	--	--	--	--	--	1	--
Stomach									
Inflammation; subacute; muscularis; multifocal	Mild	--	--	--	--	--	--	1	--
Thymus									
Mineralization; medullary, multifocal	Present	--	--	--	--	--	--	--	1
Involution; diffuse	Present	1	1	--	--	2	2	3	
Tongue									
Inflammation; subacute; lamina propria; multifocal	Minimal	1	1	2	--	3	--	2	--
Ulcer; focal		--	--	--	--	--	--	--	1

Microscopic Findings		0		5 mg/kg		16 mg/kg		50 mg/kg	
		M	F	M	F	M	F	M	F
	Group Size:	3	3	3	3	3	3	3	3
	Grade	Terminal							
Urinary bladder									
Aggregate(s); lymphoid; lamina propria; focal	Minimal	--	--	1	--	--	--	1	--
Aggregate(s); lymphoid; lamina propria; focal	Minimal	--	--	1	--	1	--	1	--
Prostate									
Inflammation; granulomatous; urethra; muscularis; focal	Minimal	--	--	--	--	--	--	1	--
Thyroid glands									
Infiltrate(s); lymphoid; focal	Minimal	--	--	1	2	--	--	--	--
Infiltrate(s); lymphoid; multifocal	Minimal	--	--	--	--	--	--	1	--
Cyst(s)	Present	--	--	1	--	--	--	--	1

Bone Histopathology

A separate histopathological evaluation of bone growth was included in this study. The results of the gross findings were not available to the Study Pathologist. The femur growth plates and stifle joints with synovial tissue were collected from animals at the time of necropsy and examined histologically at another site. This report was signed by the examining pathologist and included a signed QA statement. No findings were reported for the stifle joints. Findings in the epiphyseal growth plates included thickening at doses ≥16 mg/kg and osteochondrapathy (abnormal ossification with cartilage cell retention) at doses ≥ 5 mg/kg. Severity of findings increased with increasing dose.

Table 13: Bone Histopathology

39-Week Males

Group / Animal Number	Epiphyseal Growth Plate Finding
1 / 10123	Prominent - Normal
1 / 10124	Ossification, minimal - Normal
1 / 10125	Ossification, mild - Normal
2 / 10132	Osteochondropathy, minimal
2 / 10133	Normal
2 / 10134	Ossification, minimal - Normal
3 / 10141	Thickening, mild; Osteochondropathy, mild
3 / 10142	Osteochrondropathy, mild
3 / 10143	Osteochondropathy, mild; Ossification, minimal
4 / 10150	Osteochondropathy, moderate
4 / 10151	Thickening, mod.; Osteochondropathy, mod.
4 / 10152	Osteochondropathy, minimal

39-Week Females

Group / Animal Number	Epiphyseal Growth Plate Finding
1 / 10129	Ossification, marked - Normal
1 / 10130	Prominent - Normal
1 / 10131	Prominent - Normal
2 / 10138	Ostoechondropathy, minimal
2 / 10139	Osteochondropathy, minimal
2 / 10159	Normal
3 / 10147	Osteochondropathy, mild
3 / 10148	Thickening, mild; Osteochondropathy, mild
3 / 10149	Osteochondropathy, mild
4 / 10156	Osteochondropathy, minimal
4 / 10157	Thickening, mild; Osteochondropathy, mild
4 / 10158	Thickening, mod.; Osteochondropathy, mod.

Mod=Moderate

(Excerpted from the Applicant's submission)

Toxicokinetics

PK data of noncompartmental analysis for weeks 1, 12, and 39 is presented in Table 14. In general, PK behavior of ramucirumab was nonlinear, mean AUC_{inf} increased in a greater than dose proportional manner, Cmax behaved similarly, and the half-life increased with increasing dose at all time points tested.

Table 14: Toxicokinetic Parameters

Dose→	5 mg/kg			16 mg/kg			50 mg/kg		
Week→	1	12*	39	1	12*	39	1	12*	39
C_{max}	123	68	52	452	325	451	1,532	1,478	2,148
AUC_{inf}	10766	4770	10416	40634	16009	31297	133433	136840	233962
$T_{1/2}$ (h)	70.4	32.3	43	82.2	33.6	47	106.2	68.8	83

*3 female animal/dose, sacrificed on 85

In immunogenicity testing, 5 of 7 animals in the 5 mg/kg group tested positive for anti-ramucirumab antibodies, 3 of 9 animals in the 16 mg/kg group tested positive for anti-ramucirumab antibodies, and none tested positive for anti-ramucirumab antibodies in the 50 mg/kg group, suggesting that high levels of ramucirumab present at the high dose level may have been masking the detection of anti-drug antibodies. Despite the presence of ADA, exposure to ramucirumab was still sufficient.

7 Genetic Toxicology

In accordance with ICH S6, the Applicant did not submit genotoxicity studies; routine tests for genotoxicity are not applicable to biotherapeutics as it is not expected that monoclonal antibodies will react with DNA or other chromosomal material.

8 Carcinogenicity

In accordance with ICH S9 the Applicant did not conduct carcinogenicity studies with ramucirumab because these studies are not warranted to support marketing for therapeutics intended to treat patients with advanced cancer.

9 Reproductive and Developmental Toxicology

The Applicant did not conduct reproductive and developmental toxicity studies, stating that scientific and regulatory developments have made embryo-fetal toxicity study of ramucirumab unwarranted. Prior to submission of the BLA, the applicant had discussed the need for an embryofetal development study conducted in monkeys (the only relevant species) in order to support the application. The Applicant was advised that in this case FDA might accept an assessment of ramucirumab's developmental and reproductive toxicology that does not include ramucirumab-specific animal studies, but that if the Applicant decided to take this approach then the assessment should include relevant data and information (including non-product-specific published literature) generally explaining the effects, with respect to fetal development, of the inhibition of the VEGF pathway, along with any relevant data from studies using the surrogate murine anti-VEGF antibody (DC101) developed by the Applicant as supportive data.

The Applicant has cited ICH S9 ("Nonclinical Evaluation for Anticancer Pharmaceuticals", March 2010), to support the regulatory development; which stipulates the following points:

- Embryo-fetal toxicity studies are "not considered essential for the purpose of marketing applications for pharmaceuticals that …belong to a class that has been well characterized as causing developmental toxicity."

- "A study of fertility and early embryonic development is not warranted to support clinical trials or for marketing of pharmaceuticals intended for the treatment of patients with advanced cancer. Information available from general toxicology studies on the pharmaceutical's effect on reproductive organs should be used as the basis of the assessment of impairment of fertility."
- "A pre-and postnatal toxicology study is generally not warranted to support clinical trials or for marketing of pharmaceuticals for the treatment of patients with advanced cancer."

In addition, the Applicant cited scientific literature on the biology of the molecular target of ramucirumab, VEGFR2, and the role of the endogenous ligand, VEGF, in angiogenesis as it pertains to embryo-fetal development to support the claim that ramucirumab specific embryo-fetal toxicity studies in animals are not necessary for the assessment of embryofetal developmental risk. The literature evidence submitted includes experiments using genetically engineered animals in which VEGF-signaling is disrupted ("knockout" mice), or antibodies against VEGF or its receptors. The literature evidence is reviewed below and a sample of references included.

9.1 Fertility and Early Embryonic Development

The studies of Zimmerman et al, in mice (2001[3]) and Rhesus monkeys (2002[4]) demonstrated that pretreatment of immature female mice with DC101 (monoclonal antibody against VEGFR2) prior to induction of superovulation with PMSG/hCG inhibited luteal angiogenesis, suggesting that that VEGF acting through VEGFR2 has a role in luteal angiogenesis and corpus luteum formation. In Rhesus monkeys, 5-daily IV injections of p1C11 (another antibody against VEGFR2) led to a significant decline in Inhibin B and estradiol levels with increases in FSH and LH (pre-ovulatory). Administration of the antibody also lengthened the follicular phase from 10–12 days in the two control cycles preceding antibody administration to 20–42 days during treatment cycles. The data suggested that the recruitment-selection process of follicles in the early follicular phase in rodents or nonhuman primate is controlled by VEGF, through the VEGFR2

The Applicant cited several other authors whose studies have demonstrated the importance of VEGFR2-mediated effects of VEGF on the corpora luteal development and endocrine function, development and maintenance of endometrial and placental vascular function, successful blastocyst implantation, maternal and feto-placental vascular differentiation and development during early pregnancy, in rodents and nonhuman primates. Thus, VEGF is a critical mediator of angiogenesis that occurs across several stages of the female reproductive cycle, including the maintenance of pregnancy.

In the 39-week toxicity study in monkeys dose-dependent increases in multifocal follicular mineralization were observed, though the pathologist did not classify the severity of this finding, suggesting that it was not considered a significant drug-related

[3] Zimmerman et al (2001) *Microvascular Res* 62, 15-25 (2001)

[4] Zimmerman et al (2002) *Endocrinology* 143:2496–2502

finding. This finding may be a pharmacological consequence of the inhibition of VEGFR2 signaling with ramucirumab.

9.2 Embryonic Fetal Development

Reviews by Haigh (2008)[5] and Crivellato (2011)[6] discuss the essential of role VEGF in many different aspects of cardiovascular development, including endothelial cell differentiation, migration, and survival as well as heart formation and hematopoiesis. The VEGF-mediated assembly of a functional vasculature is also a prerequisite for the proper formation of other organs and for tissue homeostasis, because blood vessels deliver oxygen and nutrients and vascular endothelium provides inductive signals to other tissues. Thus, VEGF and its high affinity receptors VEGFR1 and VEGFR2 are critical for physiological angiogenesis, and the development of the embryonic vasculature.

Mice deficient in Flk-1 (VEGFR2) or Flt-1 (VEGFR1) were generated by gene disruption using homologous recombination in embryonic stem (ES) cells [Shalaby et al,(1995)[7], and Fong et al (1995) [8]]. Studies showed that showed that embryos homozygous for loss-of-function mutations in the gene for either receptor died in utero. Embryos homozygous for mutations in *flk-1* died in utero between 8.5 and 9.5 days post-coitum, as a result of an early defect in the development of hematopoietic and endothelial cells. In Flk-1 deficient embryos, yolk-sac blood islands were absent, organized blood vessels could not be observed in the embryo or yolk sac at any stage, and hematopoietic progenitors were severely reduced. These observations suggest that the VEGFR2 signaling pathway is essential for yolk-sac blood-island formation and vasculogenesis in the mouse embryo.

In a paper by Pauli et. al.[9] the importance of VEGFR2 signaling in pregnancy was explored using a murine anti-VEGFR2 antibody, DC101. The investigators found that administration of a single dose of the anti-VEGFR2 antibody to pregnant CD1 mice at either preimplantation (GD 3.5) or postimplantation (GD 6.5) timepoints resulted in total pregnancy loss. This study showed that the loss of pregnancy observed was due to decreases in the size and function of corpora lutea. When pregnant ovarectomized mice were supplemented with progesterone, administration of DC101 did not result in total loss of pregnancy. Thus, inhibition of VEGFR2 in this model showed a critical role for this pathway in the maintenance of pregnancy through maintenance of maternal hormone levels. The Applicant cited additional data showing that VEGF signaling has roles in implantation and placental vascular differentiation as well, added support for the critical role of the pathway in the maintenance of pregnancy.

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5 Haigh, JJ (2008) *Organogenesis* 4: 247-256;
6 Crivellato, E (2011) *Int. J. Dev. Biol.* 55: 365-375
7 Shalaby, F et al (1995) *Nature* 376:62-66
8 Fong, G, et al (1995) *Nature* 376:66-70
9 Pauli, SA et. al (2005) *Endocrinology* 146(3):1301-1311

Given that ligand(s) other than VEGF might activate VEGFRs, Carmellet et al (1996)[10] and Ferrara et al (1996)[11] assessed the role of VEGF directly, by disrupting the VEGF gene in embryonic stem cells. They found that the loss of even a single VEGF allele in mice resulted in embryonic lethality between Days 11 and 12. In these studies, angiogenesis and blood-island formation were impaired, resulting in severe developmental anomalies, including poorly developed and unsegmented branchial arches in the cranial region, unsegmented forelimb buds, a significantly underdeveloped forebrain region, developmentally delayed common atrium and primitive ventricle in the heart region, rudimentary dorsal aortae, and markedly decreased thickness of the ventricular wall. VEGF has several splice variants that result in isoforms with modest distinct differences in functional characteristics. The most common variant in mice and humans is $VEGF_{165}$ ($VEGF_{164}$ in mice). In mice engineered to exclusively express the $VEGF_{120}$ isoform, a VEGF isoform with reduced heparin binding capacity relative to the $VEGF_{164}$ and $VEGF_{188}$ isoforms, a high lethality rate occurred shortly after delivery, with surviving animals dying within 2 weeks. The mice that briefly survived presented with impaired myocardial contractility, heart enlargement, and defective angiogenesis leading to ischemic cardiomyopathy [Carmellet et al (1999)][12]. Finally, ramucirumab is an IgG1 monoclonal antibody. As this subtype is known to be transported across the placental barrier through interactions with the FcRn receptor, ramucirumab does have the potential for direct fetal exposure, especially at late stages of pregnancy.

9.3 Prenatal and Postnatal Development

In newborn mice in which most organs are already developed but kidneys are still developing, administration of a neutralizing antibody to $VEGF_{165}$, resulted in normal growth, but systemic edema was observed. Vessel formation in the renal cortex and nephron development were impaired, and abnormal glomeruli were observed in the antibody-treated mice (Kitamoto, et al, 1997)[13]. In an investigation of the role of VEGFR2 on postnatal lung development, DC101 (the rodent surrogate of ramucirumab) caused a dose- and age-dependent impairment of postnatal alveolar growth. Mice that received 3 doses of DC101 antibody every other day in the perinatal period (Days 2, 4, and 6 of life), had impaired alveolar growth at 1 and 2 weeks of age, areas of marked hemorrhage and macrophage infiltration and high mortality. Mice that received only 2 doses of DC101 antibody in the perinatal period (Days 2 and 4) had impairment of alveolar growth at 1 week of age that was reversible by 2 weeks of age (McGrath-Morrow et al, 2005)[14]. The results of studies suggest that VEGF signaling is an essential molecule for postnatal kidney and lung development.

[10] Carmellet, P. et al (1996) *Nature* 380:435-439
[11] Ferrara, N. et al (1996) *Nature* 380:439-442
[12] Carmellet, P. et al (1999) Nature Med 5:495-502
[13] Kitamoto, Y. et al (1997) *J. Clin. Invest.* 99:2351–2357
[14] McGrath-Morrow, SA., et al (2005) *Am J Resp Cell Mol Biol* 32:420-427

10 Special Toxicology Studies

Wound healing

Study #: 20018776

Study Title: A Single Dose (Intravenous) Assessment of IMC-1121B in an Incisional Model of Wound Healing in Cynomolgus Monkeys

Testing Facility: (b) (4)

Given that VEGFR inhibitors have a tendency to delay wound healing, the Applicant was asked to conduct a study in cynomolgus monkeys to determine the effect of IMC-1121B on an incisional model of wound healing after administration of a single intravenous dose. In addition, the pharmacokinetic profile of IMC-1121B was determined.

The Applicant conducted a GLP-compliant wound healing study in cynomolgus monkeys. 4 male monkeys/group were administered a single dose of 0, 5, 15, or 50 mg/kg of ramucirumab by intravenous infusion on Day 1.

Experimental Design

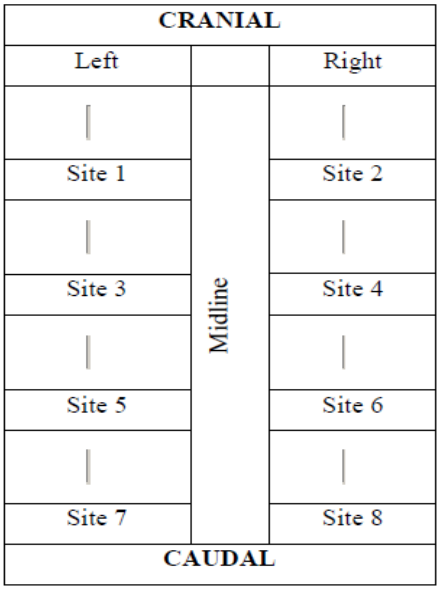
Group No.	No. of Animals[a] Male	Test Material	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)
1 (Control)	4	Control Article	0	0	5.0
2 (Low)	4	IMC-1121B	5	1.0	
3 (Mid)	4	IMC-1121B	15	3.0	
4 (High)	4	IMC-1121B	50	10.0	

Conc. = concentration
[a] Animals will be released from study on Day 25.

(Excerpted from Applicant's submission)

On Day 2 (approximately 24 hours after administration of ramucirumab or vehicle), a sterile micro-fine surgical scalpel was used to make 8 incision wounds, approximately 2 cm long by 3 mm deep (full thickness skin), on the back of each animal. Four incisions were made on each side of, and parallel to the midline, approximately 3 cm apart, as seen in Figure 29 below.

Figure 29: Schematic representation of incisional wounds



(Excerpted from Applicant's submission)

Two wounds on each animal were examined at each timepoint (Days 4, 8, 15, and 22) so that a total of 8 wounds were examined on each day. Prior to biopsy sample collection, the incision sites were observed and scored for severity of erythema and swelling (severity: 0 = none, 1 = mild, 2 = moderate, 3 = marked) as well as for full closure. In addition, the length of the incision that was closed was determined as a percentage closure based on the initial incision length. Skin biopsy samples were collected from the animals on Days 4, 8, 15, and 22 (2 skin biopsy samples per incision site = 16 total for each animal), following sedation with ketamine and Dexdomitor®. Buprenorphine (0.03 mg/kg, IM) was administered prior to the biopsy collections. The skin biopsy area was cleansed with an antiseptic and a full-thickness punch biopsy of the skin and subcutaneous tissue (approximately 5 to 8 mm skin punch biopsy each) was collected from 2 incision sites. The biopsies were placed in 10% neutral buffered formalin. The skin biopsy collection sites were closed with sutures. Buprenorphine (0.03 mg/kg, IM) was administered approximately every 8-12 hours for 3 days following each set of biopsy sample collection. Antisedan® (reversal agent for Dexdomitor®) was administered following the skin biopsy collection. The animals were allowed to recover in their cages.

A summary of the histopathological assessment of wound healing is presented in Table 15. Skin samples were semi-quantitatively scored on a severity scale of 0-4 (0=no effect, and 4=marked effect) for the features of inflammation, necrosis, granulation tissue fibroplasia, epidermal hyperplasia, and increased blood vessels (capillaries) adjacent to or within the incision site. Features with more than a whole severity score difference between controls and animals given IMC-1121B were considered outside the normal variation of the experimental model.

Table 15: Summary of histopathologic scoring of incisional wound healing process

Group Mean Histological Scoring by Incision Day – Incision Day 4				
Dose Level	0 mg/kg[a]	5 mg/kg[a]	15 mg/kg[a]	50 mg/kg[a]
Neutrophilic Inflammation Group Mean	1.3	1.0	2.0	1.1
Mononuclear Cell Inflammation Group Mean	0.9	0.5	0.1	0.9
Granulation Tissue Formation Group Mean	0	0	1.4	0
Epidermal Hyperplasia Group Mean	2.1	2.6	2.4	2.5
Fibroplasia Group Mean	0.3	0	0.3	0.5
Necrosis Group Mean	0.3	0.1	0.3	0
Wound Surface Closed (C) or Still Open (SO)	2 C 25% 6 SO 75%	1 C 12% 7 SO 88%	1 C 12% 7 SO 88%	0 C 0% 8 SO 100%
[a] n = 8				

Group Mean Histological Scoring by Incision Day – Incision Day 8				
Dose Level	0 mg/kg[a]	5 mg/kg[a]	15 mg/kg[a]	50 mg/kg[a]
Neutrophilic Inflammation Group Mean	0.4	0.1	0.6	0.4
Mononuclear Cell Inflammation Group Mean	1.6	1.5	0.9	1.4
Granulation Tissue Formation Group Mean	0	0	0.3	0.3
Epidermal Hyperplasia Group Mean	2.8	2.9	2.8	2.8
Fibroplasia Group Mean	1.8	2.0	1.8	2.0
Necrosis Group Mean	0	0	0	0
Wound Surface Closed (C) or Still Open (SO)	C = 88% SO = 12%	C = 100% SO = 0%	C = 88% SO = 12%	C = 88% SO = 12%
[a] n = 8				

Group Mean Histological Scoring by Incision Day – Incision Day 15				
Dose Level	0 mg/kg[a]	5 mg/kg[b]	15 mg/kg[a]	50 mg/kg[a]
Neutrophilic Inflammation Group Mean	0	0	0	0
Mononuclear Cell Inflammation Group Mean	2.1	1.6	1.3	1.8
Granulation Tissue Formation Group Mean	0	0	0	0
Epidermal Hyperplasia Group Mean	2.3	2.9	2.4	2.4
Fibroplasia Group Mean	2.0	2.4	2.6	2.3
Necrosis Group Mean	0	0	0	0
Wound Surface Closed (C) or Still Open (SO)	C =100% SO = 0%	C =100% SO = 0%	C =100% SO = 0%	C =100% SO = 0%
[a] n = 8 [b] n = 7				

Group Mean Histological Scoring by Incision Day – Incision Day 22				
Dose Level	0 mg/kg[a]	5 mg/kg[a]	15 mg/kg[a]	50 mg/kg[a]
Neutrophilic Inflammation Group Mean	0	0	0.1	0
Mononuclear Cell Inflammation Group Mean	1.6	1.8	1.1	1.8
Granulation Tissue Formation Group Mean	0	0	0	0
Epidermal Hyperplasia Group Mean	2.4	2.6	2.6	2.4
Fibroplasia Group Mean	2.5	2.8	2.8	2.9
Necrosis Group Mean	0	0	0	0
Wound Surface Closed (C) or Still Open (SO)	C = 100% SO = 0%	C = 100% SO = 0%	C = 100% SO = 0%	C = 100% SO = 0%
[a] n = 8				

(Excerpted from the Applicant's submission)

Pharmacokinetics

The pharmacokinetic parameters of IV infused IMC-1121B on Day 1 in the serum of male cynomolgus monkeys with incisional wounds are summarized in Table 3. The Cmax was generally observed at 0.5 or 2 hours post end of infusion. Exposure (C_{max} and $AUC_{0\text{-}inf}$) increased in approximately dose proportional manner from 5 to 15 mg/kg, but the increase $AUC_{0\text{-}inf}$ at 15 mg/kg was 16.4x the low dose, more than dose proportional. The observed V_d indicated that IMC-1121B did not significantly distribute beyond the vasculature.

Table 16: PK parameters

Group	Dose (mg/kg)	C_{max} (g/mL)	$AUC_{0\text{-}inf}$ (u.h/mL)	V_d(mL/kg)	T_{max}(h)	$T_{1/2}$(h)
2	5	144	11,025	35.3	0.5	53.9
3	15	363	34,719	36.2	0.5	58.9
4	50	1298	180,678	59.2	2	148

On Day 4, two of the 8 wounds examined among the 4 control animals were fully closed versus none of the 8 wounds examined among the 4 high dose animals; however, there were no other differences in full closure between treatment groups on Days 8, 15, or 22. No clear significant histological differences were observed in the healing process of the incisional skin wounds between control animals and animals treated with IMC-1121B at any timepoint during the study.

Study #: IM1025

Study Title: Tissue Binding Study of IMC-1121B-FITC With Normal Human and Cynomolgus Monkey Tissues

This study was completed to help support the justification for the use of the cynomolgus monkey as an appropriate animal model for toxicological assessment of ramucirumab and to identify possible tissue binding sites for the antibody. Tissue samples from human and cynomolgus monkeys (3/tissue for human, 2/tissue for monekey) were stained with ramucirumab at 2 concentrations.

As expected, the test article stained vascular endothelium in tissues from both species. There was also staining in resident macrophages from several organs. In monkeys only, positive staining of reticuloendothelial cells occurred. Both species had low levels of staining in the retina.

CNS staining was also noted in the following tissues: meninges of the brain (cerebrum-monkey only, cerebellum-human only), spinal cord (human only), perineum of the peripheral nerve (human only), perineum of the optic nerve (monkey only), choroid plexus epithelium (monkey only, apical membrane). Staining was also detected in

vascular smooth muscle of the lung (human only). Staining in all of these tissues was granular cyotoplasmic staining.

In both species there were positive findings in alveolar epithelium and gland epithelium of the salivary gland. Staining of choroid plexus epithelium (apical membrane only) was also detectable in monkeys.

Overall, the pattern of staining was similar between the two species.

11 Integrated Summary and Safety Evaluation

Ramucirumab is a recombinant human immunoglobulin G, subclass 1 (IgG1) monoclonal antibody (MAb) against human vascular endothelial growth factor receptor 2 (VEGFR2), also known as human kinase domain-containing receptor, or KDR.
The Applicant conducted a series of in vitro and in vivo studies to characterize IMC - 1121B (ramucirumab). These studies demonstrated that ramucirumab can bind to VEGFR2 and displace its ligands, VEGF-A (IC_{50}=2.3 nM), VEGF-C (IC_{50}=0.7 nM) or VEGF-D (IC_{50}=0.3nM). Ramucirumab did not to cross-bind to VEGFR1, VEGFR-3, EGFR, or to the mouse homologue, Flk-1. Moreover, in an in vivo model of angiogensis using mice subcutaneously implanted with a mix of human endothelial progenitor cells and adipose-derived stem cells in a matrigel matrix, ramucirumab demonstrated an antiangiogenic effect as measured by decreased hemoglobin and a reduction in vascular density in implants from mice treated with ramucirumab compared to a control antibody. The results of these studies support the ability of ramucirumab to bind specifically to VEGFR2, inhibit the binding of its ligand, and prevent functional responses to VEGFR2 signaling such as angiogenesis.

Since ramucirumab is not cross-reactive in mouse, its antitumor activity and anti-angiogenic mechanism of action could not be studied in the mouse. A mouse surrogate antibody, DC101, was developed for use in in vivo studies. DC101 was shown to bind specifically to Flk-1 but not to human VEGFR2.

The Applicant conducted numerous studies with DC101 in murine xenograft models of human cancers to provide nonclinical proof-of-concept that treatment with an anti-VEGFR2 antibody such as ramucirumab can inhibit the growth of a number of tumors derived from different tissues. Tumor models used in these studies included gastric, hepatic, breast, non-small cell lung, colorectal, and leukemia lines derived from human cancers. The results of the majority of these studies showed that DC101 alone or in combination with other antibodies (such as PDGFR, EGFR or VEGFR1) or other chemotherapeutic agents (such as docetaxel, Sutent or gemcitabine) significantly inhibited tumor growth. Most relevant to the current application were studies conducted using gastric adenocarcinoma models. DC101 alone significantly inhibited the in vivo tumor growth of both NCI-N87 and MKN-45 gastric carcinoma cells. In addition, DC101 administered as a monotherapy inhibited 10 of 17 human primary gastric tumor fragments implanted into athymic mice.

The Applicant submitted 5-week and 39-week GLP-compliant toxicity studies in cynomolgus monkeys. In the 5-week study, ramucirumab at dose levels of 0, 4, 12, and 40 mg/kg was administered by intravenous infusion on Days 1, 15, 22, and 29. There were no consistent remarkable changes in hematologic or clinical chemistry parameters; however high levels of creatine phosphokinase (especially in terminally sacrificed male animals and high dose females), though not dose-related, were suggestive of muscle injury and correlated with histopathologic findings of skeletal muscle degeneration.

In the 39-week study, ramucirumab at dose levels of 0, 5, 16, and 50 mg/kg was administered by intravenous infusion weekly for 39 weeks. In terminally sacrificed animals (on Day 274), there were no ramucirumab-related clinical signs or changes in hematology. Clinical pathology analysis conducted in the 39 week study also revealed few consistent trends. High control values of creatine kinase (CK) at predose, SD 85 (males) and SD 29 (females) timepoints complicated the interpretation of the changes of this marker in treatment groups in this study, however, the >700% increase in CK in male animals at the 50 mg/kg level at the end of the study (Day 273) is likely a signal of muscle damage resulting from decreased blood and nutrient supply due to inhibition of VEGFR2 by ramucirumab. Elevated levels of CK are also possible signs of cardiac damage, though histopathological findings in the hearts of ramucirumab treated animals were limited to multifocal mononuclear aggregrates.

Ramucirumab-induced renal damage was exemplified in one male animal (#10151) at the 50 mg/kg dose level. Individual clinical chemistry findings at predose, as well as Days 29 and 83 for this animal were similar to other animals in the group as well as those of controls; however, there were findings of increased blood urea nitrogen, cholesterol, and creatinine, decreased albumin concentration, and proteinuria in clinical chemistry evaluations of this animal on SD 183 and SD 273. Similar trends in BUN, cholesterol, creatinine, and proteinuria were observed in other individual animals at the 16 and 50 mg/kg dose levels at final timepoint. These findings correlated with moderate and severe glomerulonephritis at the same dose levels, in both male and female animals. The kidney toxicity appears to be a direct consequence of chronic VEGFR2 inhibition, similar to the impaired normal development of the nephron and glomeruli seen in newborn mice following inhibition of VEGFR (Kitamoto et al., 1997)[15]. At the midterm sacrifice on Day 85, the only renal findings were of minimal chronic inflammation. The failure to detect clear evidence of kidney toxicity on Day 85 may be due to the fact that the mature kidney blood vessels are more resistant to the inhibition of VEGFR2 than developing ones. The delayed kidney toxicity suggests that prolonged inhibition of VEGFR2 by ramucirumab may be capable of causing kidney toxicity clinically. Increased findings of proteinuria have been reported in some clinical trials. Although changes in blood pressure and ECG were not detected in animals treated with ramucirumab, it is possible that kidney toxicity, as seen in animals, is a contributing factor to the adverse event of hypertension reported clinically.

[15] Kitamoto, Y. et al (1997) *J. Clin. Invest*. 99:2351–2357

Changes in the epiphyseal growth plate were observed in both 5 and 39 week studies in monkeys. In the 39 week study findings were primarily of thickening and osteochondrapathy (abnormal ossification with cartilage cell retention) at doses ≥ 5 mg/kg. Severity of these findings increased with increasing dose. Similar changes in growth plate morphology have been reported with other inhibitors of the VEGF signaling pathway.

Findings of mineralization and inflammation of gray matter as well as lymphocytic cuffing in the meninges and choroid plexus were noted as present in animals from all ramucirumab treatment groups. Mineralization or inflammation of the the thymus, mainly in female animals, and ovaries were observed at the 16 and 50 mg/kg dose levels but the severity of these findings was not graded. Severe subcutaneous hemorrhage in the skin, not at the injection site, was also reported one high dose animal. The Applicant suggests that ramucirumab is not directly responsible for this finding, but rather it is a secondary event related to severe renal toxicity. Based on the pharmacology of the drug, however, ramucirumab may have directly contributed to the severity of the finding. Finally, though significant gastrointestinal (GI) tract toxicity has been associated with inhibition of VEGF signaling, there was little evidence of GI toxicity in the ramucirumab animal studies; GI findings were limited to histopatholgic signs of moderate inflammation of some sections of the GI tract in one animal, but this may correlate with clinical reports of diarrhea

The 16 mg/kg dose of ramucirumab (AUC_{inf} 40,634 µg·h/mL in week 1), administered weekly, that caused delayed kidney toxicity in monkeys is half the recommended clinical dose 8 mg/kg (AUC_{0-337} 18,300 µg·h/mL on Day 1) with an exposure 2.2x the proposed clinical dose of ramucirumab administered every 2 weeks. Anti-ramucirumab antibodies were detected in animals treated with 5 and 16 mg/kg but not at 50 mg/kg. Given the more than dose proportional exposure to ramucirumab, it is unlikely that the anti-drug antibodies had a significant effect on ramucirumab exposure in this study. It's also possible that the skin inflammation was a response to the antidrug antibodies.

Formal embryofetal development studies of ramucirumab administered to animals were not conducted to support the safety of the antibody for clinical use. Instead, in accordance with principles described in the ICHS9 and ICHS6 guidances, the Applicant has attempted to address the requirement for an assessment of the risk of reproductive toxicity from ramucirumab using non-product specific literature. The reviewed scientific literature supports a critical role for VEGFR2 signaling for embryofetal development. First, literature reports show that the cardiovascular system (the heart, blood vessels and hematopoietic cells), is the first organ system to develop in vertebrates and is essential for providing oxygen and nutrients to the embryo and adult organs. VEGF/VEGFR signaling plays essential roles in many different aspects of cardiovascular development, including endothelial cell differentiation, migration and survival as well as heart formation and hematopoiesis. The VEGF-mediated assembly of a functional vasculature is also a prerequisite for the proper formation of other organs and for tissue homeostasis, because blood vessels deliver oxygen and nutrients and

vascular endothelium provides inductive signals to other tissues. Disruption of VEGF/VEGFR signaling and angiogenesis impairs the proper functioning and/or development of tissues critical for embryo-fetal development leading to embryo-fetal lethality and teratogenicity.

Homozygous deletion of VEGFR2 in mice resulted in total embryonic lethality by Gestational Day 9.5. These embryos showed defects in the development of hematopoietic and endothelial cells as well as absence of yolk-sac blood islands and organized blood vessels. Administration of antibodies against VEGFR2 in mice or monkeys inhibited luteal angiogenesis and corpus luteum formation. In rhesus monkeys administration of an anti-VEGFR2 antibody resulted in significant decline in hormonal levels and lengthening of the follicular phase from 10–12 days in preceeding two control cycles to 20–42 d in treatment cycles.

Loss of a single VEGF allele in mice resulted in impaired angiogenesis and blood-island formation, leading to severe developmental anomalies, including poorly developed and unsegmented branchial arches in the cranial region, unsegmented forelimb buds, significantly underdeveloped forebrain region, developmentally delayed common atrium and primitive ventricle in the heart region, rudimentary dorsal aortae, and markedly decreased thickness of the ventricular wall. Administration of a neutralizing antibody to $VEGF_{165}$, to newborn mice resulted in normal growth, but systemic edema was observed. Vessel formation in the renal cortex and nephron development were impaired, and abnormal glomeruli were observed. Mice that received 3 doses of the murine anti-VEGFR2 antibody DC101 every other day during the first week after birth, had impaired alveolar growth at 1 and 2 weeks of age, areas of marked hemorrhage and macrophage infiltration and high mortality.

Overall the scientific literature supports a critical role for VEGFR2 signaling in the maintenance of pregnancy and in embryonic vasculogenesis. Based on the data presented, disruption of this signaling pathway can lead to both loss of pregnancy and fetal abnormalities and use of an antibody inhibiting this pathway during pregnancy represents a risk to the fetus. Ramucirumab should, therefore, only be used during pregnancy if the benefits to the mother outweigh the risks to the fetus.

The data support the assertion that ramucirumab is a VEGFR2 inhibitor, with an anti-angiogenic effect. The use of its murine surrogate, DC101, provides a proof-of-concept data that treatment with an anti-VEGFR2 antibody such as ramucirumab has the potential to inhibit the growth of a number of tumors derived from different tissues, alone or in combination with other chemotherapeutics. Ongoing clinical experience seems to support these earlier assumptions based on nonclinical studies. The clinical toxicities of ramucirumab were predictable from its biology. There are no outstanding pharmacology/toxicology issues that would prevent the approval of this drug in the proposed patient population.

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/s/

GABRIEL S KHASAR
01/23/2014

WHITNEY S HELMS
01/23/2014

JOHN K LEIGHTON
01/23/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125,477 **Applicant:** Eli Lilly **Stamp Date:** 08/23/2013

Drug Name: Ramucirumab **BLA Type:** NME

On **initial** overview of the BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			The formulation to be marketed is not different from the formulation used in the toxicology studies.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	Yes		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Yes		The Applicant submitted literature references in lieu of Embryo-Fetal Development studies

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		No impurities of concern were identified at this point.
11	Has the applicant addressed any abuse potential issues in the submission?	N/A		Potential abuse is not a concern
12	If this BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A		The application is not for a switch

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _____ Yes___

If the BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None at this point

Sachia Khasar 10/04/2013

Reviewing Pharmacologist Date

Whitney S Helms 10/04/2013

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

GABRIEL S KHASAR
10/08/2013

WHITNEY S HELMS
10/08/2013