I. **Study Objectives**
   A. **Primary** – To determine the maximum tolerated dose (MTD) for inhaled rhIL-15 in dogs with lung metastases.
   B. **Secondary** – Measure immune correlates (tumor aspirates and systemic PBMCs), response rate, response duration, median survival time.

II. **Investigational Plan**
   A. **Overall Study Design – Phase I Trial; Dose Escalation**
   B. **Statistical Plan**
      Sample Size Determination: Up to 18 dogs will be enrolled in this phase 1 study. Dose escalation will follow a fixed dose modified Fibonacci method, where the dose is escalated 100, 67, 50, 40 and then 33% of the previous dose as the cohorts increase. Since an MTD of IH IL-15 has not been established in dogs, we will escalate until dose-limiting toxicities (DLTs) are observed. However, we will not escalate past dose level 6 even if MTD is not reached. A DLT will be defined as grade 3 toxicity in any category (except hematologic) according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events.
Statistical Methods: In this Phase I trial, the MTD will be defined as the highest dose level at which no more than 1/6 of the subjects develops a DLT. Three patients will be enrolled per dose level, with escalation to the next dose level if no DLT is observed. If one DLT is observed, the dose level will be expanded to a total of 6 patients, and escalation will occur if no more than one DLT is observed among the 6 patients. If 2 or more patients in any cohort experience DLT, this will be defined as the maximally administered dose, and the phase I study will be concluded or dose reduced to the previous dose level (then defined as the MTD). Dose level escalation will be determined based on DLTs observed during therapy, but DLTs will be monitored after the cessation of treatment, and dose de-escalation may occur if significant late DLTs are observed.

Subject Populations for Analyses:

C. Selection of Study Population

Number of Subjects: up to 21
Species: Canine
Breed: No Specification
Initial Age: At least 1-year-old on Day 0
Weight: At least 10kg on Day 0
Sex: Male or Female, Intact or Neutered
Origin / Source: Client-Owned Pets

Dogs participating in this study will be privately owned. Dogs will be identified by their given name and owner’s surname, as recorded in their medical records. In addition, dogs will be identified by both their assigned by their unique medical record number in VMACs, and by a study number that will consist of UCD-followed by a 4-digit sequential number starting with the number (10) e.g. UCD-IL15-101, UCD-IL15-102, UCD-IL15-103, etc.

Additionally, Dogs will be identified in Red Cap Precinct 01 Penn Medicine with a unique ID starting with 40 (for Davis Site) followed by the corresponding above three digit number 101, 102, 103, etc. Example 40101, 40102

In the event the study opens up to additional sites, the study coordinator will assign the site a two digit code. The site will establish their subjects as listed above using their given university two or three digit initials, followed by the two digit code. (e.g. UW-IL15-201, CSU-IL15-301).

Previous Treatments: Normal vaccination and general health care practices are permitted. No prior chemotherapy within 2 weeks to day 0. No prior immunotherapy or radiation therapy within the last 4 weeks.

Husbandry: Normal feeding and housing post procedures until dog is released to their owners. Normal feeding and housing as provided by individual dog owners post procedures.

D. Study Schedule – Appendix A

III. Study Implementation

A. Inclusion Criteria:
   - Dogs > 1 year
• Radiographs performed within 14 days of enrollment (Day 3) consistent with metastatic disease from cytologic or histologic confirmed osteosarcoma or melanoma.
• Adequate local control of Primary tumor (Surgery or Radiation)
• Body Weight ≥ 10 kg
• VCOG-CTCAE 1.1 performance score of 0 or 1
• CBC and Chemistry performed within 14 days of enrollment (Day 3) showing adequate orang function: HCT ≥ 25%, Neutrophil Count ≥ 2,000/μl, Platelet Count ≥75,000/μl, Creatinine ≤ ULN; bilirubin ≤ ULN; ALT ≤ ULN; AST ≤ ULN
• Urinalysis performed within 14 days of enrollment (Day 3)
• One or more lung lesion measuring at least 1cm on radiographs

B. Exclusion Criteria
• Dogs unable to undergo sedation for chest films
• Owner unwilling to administer inhaled IL-15 twice daily and/or dog unable to tolerate twice daily nebulization
• Chemotherapy within 2 weeks of day 0
• Immunotherapy or radiation therapy within 4 weeks of Day 0
• VCOG-CTCAE 1.1 performance score of 2 or higher
• Concurrent therapy (NSAIDS acceptable if needed for pain control and patient has been receiving for 2 weeks or more). Pamidronate or zoledronate also acceptable

C. Visit Descriptions
• Pre-Enrollment Screening (Day -7): Dogs will be evaluated to determine eligibility. If the dog appears to meet preliminary inclusion criteria, a formal appointment/consultation (trials service) will occur. At which time, the owners will be given detailed information on the trial, including nebulization. If they are interested, the owner will review the Informed Consent Form with trials staff and sign the document. Owners will be trained in nebulization treatments (including a demonstration with the patient) and will be sent home with saline and a nebulizer to desensitize the dog. Clients will be responsible for the cost of the physical examination and the screening CBC, blood chemistry, and urinalysis, as well as any other screening tests deemed appropriate by the attending clinician. Screening CBC/CHEM/UA performed by outside reference laboratories are acceptable if they are performed within 14 days (Day -14) of enrollment (Day 3). A copy of the histologic or cytologic diagnosis of osteosarcoma or melanoma will be recorded in the study binder and in patient chart. These tests can be done prior to or after desensitization. If the dog is eligible and is successfully desensitized, then the patient will either be scheduled for an optional FNA on Day 0 with a subsequent enrollment on Day 3 or will forgo the FNA and proceed to enrollment on Day 3. Patients requiring longer than 7 days for desensitization are still eligible, however, eligibility/screening criteria will need to adhere to study requirements and therefore may need to be repeated (at the cost of the owner) to confirm eligibility.
• Day 0 (optional): Quality of Life Form (QOL), Blood sample will be obtained for CBC, PBMCs and Serum; FNA of pulmonary lesion (see appendix), 3-view thoracic...
radiographs (if pre-enrollment rads not done within 14 days of Day 3), primary tumor measurement (melanoma only). Owner sent home with nebulizer and drug.

- **Day 3 (Enrollment)**: Physical examination, (QOL), Start nebulization with IL-15 (first dose followed by 6 hours monitoring, in hospital). Sampling catheter and serial blood sampling for PK data. **If Subject did not complete a Day 0 visit then the following will also be performed:** 3 view thoracic radiographs (if not performed within the previous 14 days), PBMC, Serum, CBC/Chem 2.

- **Day 10**: Physical examination, (QOL), CBC/CHEMS/PBMCs/Serum;

- **Day 17**: Physical examination, (QOL), Blood sample CBC/CHEMS/PBMCs/Serum; Ultrasound guided fine needle aspirate of a single pulmonary metastatic lesion (see attached protocol)
  - Owner returns nebulizer and unused drug to center

- **Day 31**: Physical examination, (QOL), Blood sample for CBC, CHEM, PBMCs/Serum; Thoracic radiographs.

- **Day 45**: Physical examination, (QOL), Thoracic Radiographs

- **Day 73**: Physical examination, (QOL), Thoracic Radiographs

- **Day 101**: Physical examination, (QOL), Thoracic Radiographs; end-of-study

- **Long Term Follow-up**: Following the Day 101 visit, patients will return every 28 days for monitoring of disease progression. A physical examination, (QOL), Thoracic Radiographs will be performed at these visits.

D. Criteria to Remove Subjects from Study Post-Enrollment

- If deemed clinically indicated by the attending clinician
- If requested by the owner
- **Progression of disease is not a specific cause for removal**

E. Biological Sample Collection, Processing, & Storage

  - **Blood**: PBMCs and Serum will be collected on Days 0, 10, 17, 31; CBC/Chemistry 2 performed within 14 days of day 0, and on days 10, 17, and 31.
  - See Appendix for PBMC/Serum Protocol and storage.
  - **Urine**: screening urinalysis only
  - **Fine needle aspirates of metastatic pulmonary nodules for RNA analyses**: Day 0 and 17 if not contraindicated in the opinion of the attending clinician.
  - See Appendix for FNA Protocol, and handling.

F. Imaging:

Three-view thoracic radiographs consistent with metastatic disease should be performed within one month of Day -7 documenting metastatic lung lesions for eligibility. Radiographs will be repeated on Days 0, 31, 45, 73, and 101

G. Data Quality Assurance

IV. Investigational Product, Drug, or Device

A. Description: Recombinant Human Interleukin-15 (IL-15)-E. Coli product is described by the NCI Drug Dictionary as,

- A recombinant agent that is chemically identical or similar to the endogenous cytokine interleukin-15 (IL-15) with immunomodulating activity. IL-15, secreted by mononuclear phagocytes (and some other cell types) following viral infection, regulates T and natural killer cell activation and proliferation. This cytokine induces activation of transcription activators STAT3, STAT5, and STAT6 via JAK kinase signal transduction pathways in mast cells, T cells, and dendritic epidermal T cells. IL-15 and interleukin-2 (IL-2) are structurally similar and share many biological activities; both may bind to common hematopoietin receptor subunits, negatively regulating each other’s activity. CD8+ memory T cell number has been shown to be regulated by a balance between IL-15 and IL-2.

- SEE SECTION D or APPENDIX FOR FULL SOP DRUG PREPARATION/CALCULATION

B. Treatment Regimen:

The objective of this clinical trial is to determine the maximally tolerated dose (MTD) of Inhaled IL-15 to client owned animals that present with lung metastasis from melanoma or osteosarcoma.

C. Method of Assigning Subjects to Treatment Groups:

Standard 3 + 3 phase I cohort design. Dose escalation rules based on three (3) dog cohorts will be used to define a well-tolerated dose. Escalation will be based on assessment of a DLT, defined as any grade 3 non-hematologic or grade 4 hematologic toxicity. There will be a one week observation period between cohort escalations. The first two dogs in any cohort can be enrolled within one week of one another, but the 3rd dog or any other subsequent dog in the cohort must have a one-week waiting period after the previous dog enrolled.

1. If DLT is seen in 0/3 dogs, the dose will be escalated.
2. If DLT is seen in 1/3 dogs, an additional (up to) five dogs will be enrolled at that prescribed dose. If no DLT are seen with the additional five dogs (DLT 1/6), escalation may continue to the next higher dose
3. If DLT is seen in 2 or more dogs (2/2, 2/3, 2/4, 2/5 or 2/6) dogs within a group, the MTD will be defined as the dose administered in the cohort below.

The starting dose of IL-15 in the first cohort will be 10ug

D. Preparation and Administration of Investigational Product:

SEE APPENDIX FOR FULL SOP:
rhIL-15 Stock = 510µg/mL in solution of 25mM Sodium Phosphate, 500mM Sodium Chloride, pH 7.4. 1mL volume per vial. Stored at -80°C. The IL-15 will be thawed per dosing cohort (See chart).

Volume chart for making up 90 or 100mL of IL-15 final working concentrations with 0.01% CSA and 0.9% Saline.

<table>
<thead>
<tr>
<th>DOSE LEVEL</th>
<th>Dose Escalation</th>
<th>Actual Dose</th>
<th>Actual IL15 Conc.</th>
<th>Saline Vol. to Discard</th>
<th>1% CSA to Add</th>
<th>Stock 1L-15 (510µg/mL) to Add</th>
<th>Vials of IL-15 to Thaw</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>---</td>
<td>10ug</td>
<td>3.333 µg/mL</td>
<td>1.7 mL</td>
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<td>16.4 mL</td>
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Dispensing:

Clinical trials will be responsible for thawing and mixing the IL-15 and pulling up into 3mL syringes. The pharmacy will create a label. The script will be printed by the pharmacy and include:
- **Refrigerate** place 3mL of prepared drug into the nebulizer well. Administer twice a day over 10-15 minutes until vapor is no longer seen, as demonstrated for owner, for 14 days. IL-15 will need to be packaged on ice for transport with care to avoid freeze/thaw.
E. Blinding of Study Intervention:
All dogs will receive the drug. Study is unblinded.

F. Prior and Concomitant Therapy:
Bisphosphonates allowable. NSAIDS allowed if patient requires for pain control and has been on for greater than 2 weeks. No other concurrent therapy allowed. Two week washout from chemotherapy. Four week washout from radiation therapy and/or immunotherapy. All medications will be recorded in the study CRF.

G. Storage and Handling of Investigational Product

**Manufacturer:** Biological Resources Branch; National Cancer Institute

**Storage Instructions:** Store at < -70°C

**Handling Instructions:** Once prepared, IL-15 may stored at 4°C for up to 14 days

**Dispensation Instructions:** Study medication for the nebulizer will be dispensed in 3ml syringes, for a 14 day supply. Owner will be sent home with nebulizer, tubing, nosecone and/or hood, and Personal Protective equipment including, chemo gown, dispensable N95 mask, eye protection, and gloves. Owner will be instructed to use eye protection and instructions to perform in an well ventilated area. Label will be printed to include *Refrigerate* place 3ml of prepared drug into the nebulizer well. Administer twice a day am and pm (8-12 hours apart) over 10-15 minutes as demonstrated (until no vapor is seen). *See Owner handout*.

**Return or Disposition Instructions:** On Study Day 17, the owner will be instructed to return the nebulizer, hose, safety equipment, and any unused drug, and all empty syringes for proper disposal. In the event the subject is not able to return for future visits, the owner will be given a FEDEX label with instructions for returning the machine.

V. Investigational Requirements

A. Informed Consent

All owners must read and sign the Owner Informed Consent Document (Appendix B) prior to enrollment of their pet into the study. A copy of the signed consent form will be provided to the owner, one copy will be scanned in to the patient medical record in VMACS on the visit for Day 0 and the original signed copy will be kept in the patient study binder.

B. Adverse Events / Safety Assessment

**Definition of an Adverse Event:** Any grade 1 or higher toxicity identified by VCOG criteria.

**Method of Evaluating and Recording Adverse Events:** Adverse events will be recorded and documented in the binder CRF forms.

**Required Adjustments if Adverse Events Occur**

- *Reporting Requirements for Serious Adverse Events: In the event of a serious adverse event, the Principal
investigator will be notified. If 2 or more serious adverse events at a grade 3 toxicity in any category (except hematologic) are identified in a cohort, the cohort will be closed.

- Unblinding Procedures: N/A
- Stopping Rules if at anytime the protocol is not being followed, it will be reported to the IACUC. Any grade 3 or 4 toxicities, except for in the event for transient neutropenia/thrombocytopenia. The study may be halted if a MTD is seen within any of the cohorts.

C. System of Data Capture:
   Paper CRF records in a study binder will be kept in Oncology.

D. Confidentiality of Data
   Identity of patients and their owners will be kept confidential in any presentations or publication of the data generated in this study.

E. Retention of Records
   Location of Records During the Study: Study binders will be kept in the office of CCAH 171 while the study continues to have active patients.
   Individual(s) with Access to Records During the Study: PI, Co-Investigators, Study Coordinators, Attending Clinical Trials Doctor
   Location of Records After Study Completion: Once all patients have completed the study, the binders will be transferred to the Principal Investigator.
### VI. Appendices

#### A. Appendix A

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Pre-Enroll Day -7</th>
<th>Patient Desensitized</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 17</th>
<th>Day 31</th>
<th>Day 45</th>
<th>Day 73</th>
<th>Day 101</th>
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<td>Inhaled IL-15 (*saline)</td>
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</table>

1 Owner is administering at home prior to appointment.

^ Long Term Follow-up Visits will occur every 28 days after the Day 28 visit.