UCDAVIS VETERINARY MEDICINE
Veterinary Center for Clinical Trials

Study Protocol

I. Overview / Rationale

The IL-15 super-agonist ALT-803 is a fusion of IL-15 and the human IL-15Rα. This has been shown to increase the biological activity of IL-15 approximately 5-fold with corresponding increases in serum half-life and anti-tumor effects. We hypothesize that systemic/subcutaneous administration of ALT-803 in cancer-bearing dogs will mobilize and activate endogenous NK and memory T cells, which will translate to favorable anti-tumor effects against OSA and melanoma metastases.

This is designed as a rapid acceleration phase 1 trial. We will evaluate safety, toxicity, immunologic endpoints, and preliminary data for response rates in this first-in-dog study. Dogs will receive a two subcutaneous injections of ALT-803, fourteen days apart.

II. Study Objectives

A. Primary endpoints for this aim include dose limiting toxicity, and pharmacokinetic/dynamic indicators of ALT-803

B. Secondary endpoints include radiographic response to therapy, progression-free survival (PFS) and quantity, activity, and kinetics of circulating NK and memory T cells. Other secondary endpoints include evaluation of anti-ALT-803 antibodies and serum cytokines

III. Investigational Plan

A. Overall Study Design – Rapid acceleration phase I trial; Dose escalation

40% dose escalation between single-patient cohorts in the accelerated phase reverts to a standard 3+3 cohort design when one dose-limiting toxicity or two moderate toxicities (excluding minor dermatologic toxicities at injection site) are observed during any cycle.

B. Statistical Plan

1. Sample Size Determination: We will start at 30 ug/kg and proceed to 42 ug/kg, 60 ug/kg, 82 ug/kg, 115 ug/kg, and 160 ug/kg. We estimate that up to 12 patients will be enrolled. This is based on the conversion to a 3 + 3 Study design, where up to 5 dogs could be enrolled at the first dose for which a dose limiting toxicity is observed. Assuming a patient fallout rate of 20% (2 dogs), we arrived at an estimate of 14 dogs.
2. Statistical Methods: Standard rapid acceleration phase 1 MTD trial
3. Subject Populations for Analyses: Dogs with OSA or MEL that have pulmonary metastasis

C. Selection of Study Population

1. Number of Subjects: Up to 14
2. Species: Canine
3. Breed: Any
4. Initial Age: Greater than 1 year old
5. Weight: Greater than 10 kg on Day 0
6. Sex: Any
7. Origin / Source: Client-owned:
8. Identification: 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-803-1001, UCD-803-1002, UCD-803-1003, etc.

9. 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-803-**20**01, CSU-803-**30**01).
10. 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.
11. 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 – See **Error! Reference source not found.**)

IV. Study Implementation

A. Inclusion Criteria

- Dogs > 1 year
- Radiographs within 28 days, consistent with metastatic disease from cytologic or histologic confirmed osteosarcoma or melanoma.
- Body Weight ≥ 10 kg
- VCOG-CTCAE 1.1 performance score of 0 or 1
- HCT ≥ 25%
- Neutrophil Count ≥ 2,000/ul
- Platelet Count ≥75,000/ul
- Creatinine ≤ ULN; bilirubin ≤ ULN; Any other clinically significant grade 2 or higher hematologic, biochemical abnormality.
- **At least one lung lesion measuring at least 1cm on radiographs**

B. Exclusion Criteria

- Dogs unable to undergo sedation for chest films
- Owner unwilling or unable to bring patient in for required study visits
- 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)

C. Visit Descriptions

Rebhun RB, *et al. J Immunother Cancer* 2022; 10:e004493. doi: 10.1136/jitc-2022-004493

1. Pre-Enrollment (Day -7 to 0): Dogs will be evaluated to determine eligibility. Clients will be responsible for the cost of the physical examination and the screening CBC, blood chemistry, urinalysis, thoracic radiographs (blood/UA acceptable within 14 days of Day 0, radiographs within 28 days of day 0), and any other screening tests deemed appropriate by the attending clinician. Screening CBC/CHEM/UA must be performed at UC Davis. Outside reference laboratories are not acceptable. Radiographs outside of UC Davis are acceptable as long as they have been reviewed by a radiologist within UC Davis VMTH. A copy of the histologic or cytologic diagnosis of osteosarcoma or melanoma will be recorded in the study binder and in the patient chart.
2. Definition of Day 0: physical exam, owner consent, thoracic radiographs, FNA of pulmonary lesion, primary tumor measurement (melanoma only) or primary tumor radiographs (OSA only).
3. Definition of Day 3: physical exam, CBC, CHEM, PBMC, Serum. Owner Assessment, Quality of Life Form (QOL); Administration of ALT-803 #1
4. Day 7: *Physical examination*, (QOL), CBC, CHEM, PBMC, Serum, AE forms
5. Day 10: *Physical examination*, (QOL), CBC, CHEM, PBMC, Serum, AE form
6. Day 17: *Physical examination*, (QOL), CBC, CHEM, PBMC, Serum, AE form, ALT-803 #2
7. Day 21: *Physical examination*, (QOL), CBC, CHEM, PBMC, Serum, AE form
8. Day 31: *Physical examination*, (QOL), CBC, CHEM, PBMC, Serum, AE form, FNA of pulmonary lesion, UA, Thoracic rads, primary tumor measurement (melanoma only) or primary tumor radiographs (OSA only)
9. Day 45: *Physical examination*, (QOL), CBC, PBMC, Serum, AE form, Thoracic rads
10. Day 73: *Physical examination*, (QOL), CBC, PBMC, Serum, AE form, Thoracic rads
11. Day 101: *Physical examination*, (QOL), CBC, PBMC, Serum, AE form, Thoracic rads

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 <u>is not</u> a specific cause for removal**

E. Biological Sample Collection, Processing, & Storage

1. Blood: CBC/Chemistry 2 performed within 14 days of day 0. CBC, PBMC, Serum will be collected on Days 3, 7, 10, 17, 21, 31, 45, 73, 101;Chemistry 2 performed on days 3, 7, 10, 17, 21, 31. See Appendix for PBMC/Serum Protocol and storage
2. Urine: UA performed within 14 days of day 0 and on day 31.
3. Fine needle aspirates: FNA of metastatic lesion for RNA analyses on Day 0 and Day 31 if not contraindicated in the opinion of the attending clinician.

F. Imaging: Thoracic radiographs consistent with metastatic disease should be performed within one month of Day 0 documenting metastatic lung lesions for eligibility. Radiographs will be repeated on Days 0, 31, 45, 73, and 101

G. Data Quality Assurance

This study will be conducted in accordance with 21 CFR 511.1 and under the principles of Good Clinical Practices as defined in VICH GL9. (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf)

V. Investigational Product, Drug, or Device

A. Description:

ALT-803, a novel IL-15 superagonist complex consisting of an IL-15 mutant (IL-15N72D) bound to an IL-15 receptor α/IgG1 Fc fusion protein. ALT-803 has improved pharmacokinetic properties, longer persistence in lymphoid tissues and enhanced anti-tumor activity compared to native, non-complexed IL-15 in vivo. Preclinical studies have shown that ALT-803 simultaneously mobilizes both the innate and adaptive arms of the immune system to elicit rapid and durable responses against numerous cancers and virally-infected cells.

B. Treatment Regimen: ALT-803 will be given subcutaneously on days 0 and 14

C. Method of Assigning Subjects to Treatment Groups: Rapid dose escalation study. 40% dose escalation between single-patient cohorts in the accelerated phase reverts to a standard 3+3 cohort design when one dose-limiting toxicity or two moderate toxicities (excluding minor dermatologic toxicities at injection site) are observed during any cycle Escalation will be based on assessment of a DLT, defined as any grade 3 non-hematologic or grade 4 hematologic toxicity. There will be three (3) week observation period between individual cohort escalations. For the 3+3 phase, the first two dogs in any cohort can be enrolled within two weeks of one another, but the 3rd dog or any other subsequent dog in the cohort must have a two-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.

D. Preparation and Administration of Investigational Product: ALT-803 will be given subcutaneously. Sites of administration will be recorded on a body map. Different sites should be used for each injection.

E. Blinding of Study Intervention: All dogs will receive ALT-803. Study is unblinded.

F. Prior and Concomitant Therapy: No concurrent therapy allowed, NSAIDS allowed if patient requires for pain control and has been on for greater than 2 weeks. Two-week washout from chemotherapy. A 4-week washout from radiation therapy and/or immunotherapy. All medications will be recorded in the study CRF.

G. Storage and Handling of Investigational Product

1. Manufacturer: Altor Biosciences
2. Storage Instructions: ALT-803 should be stored at 4°C
3. Handling Instructions: Stable for 42 months at 4°C
4. Dispensation Instructions: Return to investigator
5. Return or Disposition Instructions: Return to investigator

VI. Investigational Requirements

A. Informed Consent

All owners must read and sign the Owner Informed Consent Document (Appendix X) 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

1. Definition of an Adverse Event: Any grade 1 or higher toxicity identified by VCOG criteria
2. Method of Evaluating and Recording Adverse Events: Adverse events will be recorded and documented in the binder CRF forms.
3. Required Adjustments if Adverse Events Occur

a) Reporting Requirements for Serious Adverse Events: In the event of a serious adverse event, the Principal investigator will be notified immediately. 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

b) Unblinding Procedures: N/A

c) 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 cohort

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

1. 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
2. Individual(s) with Access to Records During the Study: PI, Co-Investigators, Study Coordinators, Attending Clinical Trials Doctor
3. Location of Records After Study Completion: Once all patients have completed the study, the binders will be transferred to the Principal Investigator
4. Individual(s) with Access to Records After Study Completion: PI, Co-Investigators, Study Coordinators, Attending Clinical Trials Doctor

VII. Study Budgeting / Billing

A. Costs Covered by Study: Once determined eligible and the pet is enrolled, the study will cover the cost of appointment fees, blood sampling, sedation, fine-needle aspirates, thoracic radiographs, and all study related procedures, drug and administration.

B. Costs Not Covered by Study: Owners are responsible for eligibility screening which may include initial or recheck office examination, bloodwork including CBC/Chemistry panel/UA, thoracic radiographs, and confirmed diagnosis of Osteosarcoma, or Melanoma

C. Costs Covered for Adverse Events: If the pet experiences adverse event(s) as a result of taking part in this study, and is in need of medical treatment, the study sponsors will offer to pay for medical treatment for injury/side effects up to $2000. The study can ONLY pay for costs of therapy incurred at the UC Davis Veterinary Medical Teaching Hospital. Costs associated with treatment beyond $2000 will be at the expense of the owner.

VIII. Appendices

Procedure	Pre-Enroll Day -7 to 0	Visit D0	Visit D3	Visit D7	Visit D10	Visit D17	Visit D21	Visit D31	Visit D45	Visit D73	Visit D101
PE		X	X	X	X	X	X	X	X	X	X
FNA lung lesion		X						X			
CBC	X		X	X	X	X	X	X	X	X	X
Chemistry	X		X	X	X	X	X	X			
Urinalysis	X							X			
Thoracic radiographs		X						X	X	X	X
PBMCs			X	X	X	X	X	X	X	X	X
Altor-803 SQ			X			X					
Owner AE				X	X	X	X	X	X	X	X
Injection site AE				X	X	X	X	X	X	X	X
Serum (luminex)			X	X	X	X	X	X	X	X	X
QOL			X	X	X	X	X	X	X	X	X
Serum (antibody titer)			X		X	X		X	X	X	X
Primary tumor size (melanoma only)		X						X			
Primary tumor rads (OSA only)		X						X			