COTC021: Evaluation of Orally Administered mTOR inhibitor Rapamycin in Dogs in the Adjuvant Setting with Osteosarcoma

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**Précis:** Cancer cell progression requires the activation of cellular growth pathways and the inactivation of cellular death pathways. The protein mTOR occupies a key position in both of these pathways and plays an important role in progression of many cancers. Recent data suggests that inhibition of the mTOR pathway may have additional effects in targeting metastatic progression by altering the ability of highly metastatic cancer cells to endure and survive the stresses of metastasis. Accordingly, targeting the metastatic phenotype of cancers through mTOR inhibition may meet a currently unmet need for patients at risk for metastatic progression.

In a previously completed canine osteosarcoma dose escalation study (COTC003), the mTOR inhibitor, rapamycin, was safely administered as a parenteral formulation at doses ranging from 0.01 mg/kg to 0.08 mg/kg. Pharmacokinetic exposures achieved in these dogs were translatable to those seen in human patients and resulted in effective tumoral and surrogate PBMC modulation of pS6RP, a proximate target of the mTOR pathway. A follow-up study (COTC008) administering this parenteral formulation subcutaneously or intramuscularly for longer durations resulted in granuloma formation in dogs. Furthermore, the stability of this formulation was found to be limited. Nonetheless, these data suggested that rapamycin may be useful and informative as an mTOR inhibitor in dogs with cancer; and while the parenteral formulation may be useful as a scientific and discovery tool, this formulation is not a reasonable therapeutic agent for pet dogs. A small pilot study (COTC013) administering a dosage of 0.08 mg/kg either orally or intramuscularly demonstrated that rapamycin had acceptable bioavailability via the oral route when compared to intrasmuscular administration. Such oral delivery may allow long-term dosing of dogs and the future evaluation of oral rapamycin as a means to prevent metastatic progression following resection (adjuvant therapy) of the primary tumor in patients with osteosarcoma.

A confirmatory PK-driven validation study of orally-administered rapamycin performed in tumor-bearing dogs (COTC020) demonstrated translatable pharmacokinetics, utilizing both trough and dose equivalent AUC parameters achieved with daily dosing of 0.1 mg/kg in tumor-bearing dogs. Based upon observed toxicities in COTC020, a 4-day on/3-day off schedule was simulated based upon 5-day on/2-day off PK data and is the basis for the proposed study.



|  |  |  |
| --- | --- | --- |
| **Calculated blood pharmacokinetic parameters on day 26 for rapamycin following oral dosing in dogs at a dose of 0.1 mg/kg via two dosing schemes** | | |
| **Pharmacokinetic Parameter** | **M, W, F Dosing** | **M-F Dosing** |
| AUC0-48h (ng x hr x ml-1) | 90.6 ± 31.0  (55.4, 102.4, 113.9) | 436.7 ± 225.1  (177.5, 549.6, 583) |
| Cmax (ng/ml) | 6.50 ± 4.14  (2.94, 4.53, 6.1, 12.4) | 16.9 ± 7.9  (9.6, 15.8, 25.2) |
| Tmax (hr) | 2.00 ± 1.41  (1, 1, 2, 4) | 3.33 ± 1.15  (2, 4, 4) |
| C48h (ng/ml) | 0.78 ± 0.57  (0, 0.704, 1.12, 1.28) | 6.51 ± 4.05  (1.93, 7.95, 9.64) |
| Values represent mean ± SD for each parameter. The value for each animal used in calculation is shown below in parentheses. | | |

Herein we proposed an adjuvant therapy study in dogs with osteosarcoma which will undergo the Standard of Care (SOC) followed by adjuvant orally-administered rapamycin. The dose and schedule in this study are based upon the results of COTC020, and is proposed to demonstrate the anti-metastatic effects of orally-administered rapamycin which is both tolerable and approximates the exposures known to modulate mTOR in preclinical models, while achieving therapeutically relevant PK targets in humans. This study will occur contemporaneous to a SOC-only study (COTC022). Dogs will be randomized to either COTC021 or COTC022 at the time of eligibility determination. Collectively these data will be integrated within the development consideration of rapamycin and rapalogs for both canine and human pediatric sarcoma patients.

**Table of Contents:**

1.0 Study Objectives

2.0 Study Implementation

2.1 Study design

2.1.1 Standard of Care Treatment Regimen

2.1.2 Initiation of Adjuvant Rapamycin Therapy

2.2 Study patients

2.2.1 Baseline Evaluation for Eligibility

2.2.2 Eligibility Criteria

2.2.3 Exclusion Criteria

2.2.4 Patient Registration

3.0 Patient Procedures for Standard of Care

3.1 Study Schedule

3.2 Surgery

3.3 Biological Collection and Application

3.4 Carboplatin Dosing and Administration

3.5 Patient Monitoring

3.6 Carboplatin Dose Modification Guidelines

3.7 Continuation on Study

4.0 Patient Procedures for Rapamycin Administration

4.1 Rapamycin Administration

4.2 Dosing Regimen

4.3 Patient Procedures

4.4 Study Schedule

4.5 Biological Collection and Application

5.0 Clinical Evaluation

6.0 Toxicity

6.1 Drug Holidays

6.2 Study Stopping Rules

7.0 Off Study Criteria

8.0 Study Communications

9.0 Adverse Events

9.1 Reconciliation of Adverse Events and Serve Adverse Events

10.0 Necropsy

11.0 Data Reporting and Record Keeping

11.1 Reporting

11.2 Verification of C3D data entry

12.0 Study Drug Formulation

13.0 Study Drug Management

13.1 Drug Storage

13.2 Drug Administration

13.3 Drug Reconciliation and Verification

13.4 Human Contact

14.0 Future Use of Collected Data/Samples:

14.1 Biological endpoints to be assessed

**1.0 Study Objectives**

(1) Investigate the antimetastatic effects of rapamycin when added to an SOC backbone for the treatment of canine osteosarcoma, as measured by statistically significant improvement in disease-free interval (DFI) over SOC alone in a prospective, randomized clinical trial setting

(2) Identify key factors related to tolerability and clinical efficacy of rapamycin when studied in the Minimal Residual Disease (MRD) setting, such as PK parameters, evidence of target modulation within surrogate tissues, and patient/tumor related factors

**2.0 Study Implementation**

**2.1 Study Design**

An open label, prospective preclinical trial of SOC followed by orally administered rapamycin will be conducted in dogs with osteosarcoma through the Comparative Oncology Trials Consortium. This is a fixed dose and schedule study in dogs with osteosarcoma. The study period for rapamycin exposure after completion of SOC will be 4 months (Weeks 15-31). Dogs with progressive disease during or upon study completion will be removed.

**2.1.1 Standard of Care Treatment Regimen**

Based on eligibility, which requires cytologic (inclusive of alkaline phosphatase positivity) or histologic confirmatory diagnosis of appendicular osteosarcoma with no evidence of metastatic disease, dogs will undergo definitive surgery at the COTC institution to allow banking of tumor and normal tissue removed at the time of surgery. Surgery must occur within 7 days of enrollment on study. Definitive surgery is defined as removal of the entire tumor mass via limb amputation.

Between Days 10-14 post-operative, dogs must begin adjuvant carboplatin chemotherapy, unless special circumstances arise, ie. surgical dehiscence with infection. Discussion with Dr. Fan is required prior to deviation from this timeline.

Dogs will receive 4 doses of carboplatin at 300 mg/m2 given at 3 week (q 21day) intervals. Follow up visits to the COTC site are required at pre-determined intervals after completion of chemotherapy for physical examination, biologic sample collections, and thoracic radiographic examinations.

**2.1.2 Initiation of Adjuvant Rapamycin Therapy**

After completion of all 4 doses of adjuvant carboplatin, dogs must be restaged and confirmed as No Evidence of Disease (NED) based upon thorough physical examination and 3-view thoracic radiographs prior to initiation of rapamycin administration, for which a treatment period of 16 weeks is anticipated.

**2.2 Study Patients**

**2.2.1 Baseline Evaluation for Eligibility**

* The following are required for enrollment and must be performed at the COTC institution 7 days before definitive surgery:
  + Physical examination with weight recorded
  + CBC, serum biochemistry, urinalysis
  + No evidence of pulmonary metastatic disease based upon 3-view thoracic radiography reviewed by a board-certified radiologist
  + No evidence of visceral metastases based upon abdominal ultrasound reviewed by a board-certified radiologist

**2.2.2 Eligibility Criteria:**

* Histologically or cytologically (inclusive of alkaline phosphatase positivity) confirmed osteosarcoma
* Measurable disease that is amenable to surgical removal via amputation (No evidence of metastasis based upon physical exam, thoracic radiographs, and abdominal ultrasound).
* Favorable performance status: Grade 0 or 1 (*modified ECOG criteria)*
* ONLY newly diagnosed dogs are eligible with no prior therapy (conventional or metronomic chemotherapy, ionizing radiation, bisphosphonates) for osteosarcoma
* Dogs receiving analgesics including NSAIDs, gabapentin, tramadol, or other will be eligible for study inclusion
* Informed owner consent for trial (approved by IACUC)
* Dogs must undergo full post-mortem examination (necropsy) if they die while on study

**2.2.3 Exclusion Criteria:**

* Dogs < 25 kg in size
* Dogs without measurable disease (appendicular osteosarcoma) at presentation to the regional COTC site
* ANY prior therapy for osteosarcoma (conventional or metronomic chemotherapy, ionizing radiation, bisphosphonates)
* Concurrent medications deemed incongruent with this study. All pre-existing necessary medications should be recorded as concomitant medications.
* Significant co-morbid illness, which includes but is not limited to renal or hepatic failure, history of congestive heart failure or clinical coagulopathy
* Creatinine > 3.0 mg/dL
* Bilirubin > 2.0 mg/dL or elevated bile acids
* HCT < 25%, platelets < 150,000 cells/ul
* Any hematologic/biochemical abnormality > grade 1 (VCOG-CTCAE)

**2.2.4 Patient registration**

Eligibility and Enrollment eCRFs should be completed for all potentially eligible dogs and sent by email to Christina Mazcko: [mazckoc@mail.nih.gov](mailto:mazckoc@mail.nih.gov). The signed informed consent form should be faxed to Christina Mazcko (301) 480-7328 at time of enrollment. Upon confirmed enrollment, a time line for study drug shipping will be arranged. C3D enrollment eCRF completion is required before a trial package may be sent. Once confirmation of a patient’s enrollment is made, the COTC site research team can enter C3D and complete the Screening eCRFS. All Screening eCRFS must be complete prior to the first treatment administration.

**3.0 Patient Procedures for Standard of Care**

**3.1 Study Schedule**

An overview of the study schedule is provided in Table I

Table I: Study Schedule: Standard of Care Week 1-15

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Action | **Eligibility**  **7 days pre-operative** | **Week 1** | **Week 3** | **Week 6** | **Week 9** | **Week 12** | **Week 151 start rapamycin therapy** |
| Patient Eligibility | X |  |  |  |  |  |  |
| Physical Exam | X | X | X | X | X | X | X |
| Surgery |  | X |  |  |  |  |  |
| Chest radiographs | X |  |  |  | X |  | X |
| CBC/chemistry profile/UA | X |  | X | X | X | X | X |
| Abdominal ultrasound | X |  |  |  |  |  |  |
| Carboplatin administration 300mg/m2 IV |  |  | X  (1st dose) | X  (2nd dose) | X  (3rd dose) | X  (4th dose) |  |
| Serum |  | X (prior to Sx) |  |  |  |  |  |
| Whole Blood |  | X (prior to Sx) |  |  |  |  |  |
| PBMC |  | X (prior to Sx) |  |  |  |  |  |

1Rapamycin therapy must be initiated within 7 days following the Week 15 recheck

**3.2 Surgery**

Following the appropriate baseline imaging and blood collections and confirmation of eligibility, dogs will undergo surgery within 7 days at the COTC institution. At the time of surgery, tumor and normal tissue samples will be collected and stored according to a standardized SOP (SOP04). Lymph nodes (prescapular and axillary for forelimb osteosarcoma lesions and popliteal for hindlimb osteosarcoma lesions) must be assessed by histopathology to ascertain if lymph node metastasis exists. Dogs with confirmed regionally nodal metastatic disease will be removed from study. A report of the surgical approach/methods should be sent as a PDF to Christina Mazcko to be added to the database for each patient.

**3.3 Biological Collections and Applications**

Tumor and normal tissue will be collected during surgical amputation. Serum, whole blood, and PBMC will be collected at baseline prior to surgery (SOP 01, 02, 03).

**3.4 Carboplatin Dosing and Administration**

Between 10-14 days post-surgical amputation, dogs will begin adjuvant carboplatin chemotherapy at the COTC institution. The COTC clinician will obtain a CBC, chemistry profile and urinalysis within 48 hours of planned carboplatin administration. The lab results will be reviewed and the dog deemed acceptable to receive treatment. The dose of carboplatin will be 300 mg/m2 given as an inpatient IV infusion. For this study, dogs must have a minimum of 2,000 neutrophils/uL and 150,000 platelets/uL with normal renal function as determined by BUN/creatinine to safely receive chemotherapy.

Carboplatin will be formulated for administration based on each COTC site’s conventional practice according to the study schedule. The starting carboplatin dose should be 300 mg/m² unless the COTC treating clinician has concerns re: patient obesity or other factors, in which case a consensus for an alternate starting dose will be reached between Dr. Fan and COTC site PI.

**3.5 Patient monitoring**

Dogs will be discharged from the hospital on the day of carboplatin administration and owners will be instructed to contact the COTC site if any unusual/adverse events occur once a patient is discharged. If adverse events are seen, the patient should return immediately for evaluation. Owners will complete the Owner Assessment Form to record their impressions of their dogs’ clinical status and during the study period. Owner Assessment Forms will be submitted for review at each visit during the study period.

**3.6 Carboplatin Dose Modification Guidelines**

**Dose delay**: In the event that a dog has unacceptable labwork to allow for safe chemotherapy administration (e.g. Grade 1 or higher neutropenia [less than 1,500 cells/ul], thrombocytopenia [less than 100,000 cells/ul]), the COTC clinician should prescribe a dose delay of up to 7 days and repeat any pertinent labwork prior to re-attempting carboplatin administration. Delays in treatment should be kept to ≤ 7 days in order to maintain dose intensity while managing toxicity and protecting patient safety. Confirmation of the timeline and plan for the patient in question should be communicated prior to implementation with Dr. Fan and/or Christina Mazcko.

**Dosing modification after a treatment delay:** In dogs with recurrent treatment delays due to Grade 2 or higher myelosuppression (neutropenia [less than 1,499 cells/ul] or thrombocytopenia [less than 99,000 cells/ul]), a 10% reduction in carboplatin should be prescribed for the ensuing cycle, but should maintain the q21 treatment interval. Confirmation of the timeline and plan for the patient in question should be communicated prior to implementation with Dr. Fan and/or Christina Mazcko.

**3.7 Continuation on Study**

After completion of chemotherapy (Week 12), dogs must return to the COTC member site for re-evaluation (Week 15) and determination if they are still free of disease based upon thorough physical examination and 3-view thoracic radiographs and can continue on to receive rapamycin (Investigational arm) or receive no additional treatment (SOC arm) based upon prior study arm randomization.

**4.0 Patient Procedures for Rapamycin Administration**

**4.1 Rapamycin Administration**

Following thorough physical examination, 3-view thoracic radiographs, and blood collections, dogs will initiate rapamycin therapy no later than 7 days following Week 15 reevaluation. In context of rapamycin treatment cycles, dogs will receive oral rapamycin initially on Day 1 at the COTC site. Dogs will subsequently receive oral rapamycin at home (via owner administration) during study participation. Pet owners will be instructed to wear disposable latex gloves when handling rapamycin tablets to minimize any risks associated with rapamycin’s immunosuppressive properties. Owners will be instructed to administer drug in the mornings after a minimum of four hours fasting and allowed to feed no sooner than one (1) hour post administration.

**4.2 Dosing Regimen**

Oral administration of rapamycin will be initiated on Mondays. Owners will be instructed to maintain a diary and adhere to a consistent dosing schedule with rapamycin being administered on an empty stomach and allowed to feed no sooner than one hour after dosing.

Dosing will occur Monday through Thursday of each week, with NO rapamycin administration on Fridays, Saturdays, or Sundays.

**4.3 Patient Procedures**

4.3.1 Oral administration of rapamycin will be initiated on Monday (Day 1). Owners will be sent home with rapamycin for the first 11 days of treatment.

* 1. 2Owners will be instructed to contact the COTC site if any unusual/adverse events occur once a patient is discharged. If adverse events are seen, the patient should return immediately for evaluation. Owners will complete the *Owner Assessment Form* to record their impressions of their dogs’ clinical status and confirm drug administration during the study period. *Owner Assessment Forms will be submitted for review at each visit* during the study period.
     1. On Day 11(Thursday) re-evaluation at the COTC member site is required prior to rapamycin administration. Dogs should be fasted prior to presentation. A baseline whole blood sample (Time 0, trough) will be collected first, and then followed by rapamycin administration in-house by the COTC study coordinators. Whole blood will then be collected for a 6-point PK analysis over 24 hours. PK samples will be shipped at the end of the study. Dogs will be discharged on Day 12 (Friday) to their owners.
     2. On Day 25 (Thursday) re-evaluation at the COTC member site is required prior to rapamycin administration. A CBC, chemistry profile, and UA will be collected and submitted in-house. Dogs should be fasted prior to presentation. A baseline whole blood sample (Time 0, trough) will be collected first, and then followed by rapamycin administration in-house by the COTC study coordinators. Whole blood will then be collected for a 6-point PK analysis over 24 hours. PK samples will be shipped at the end of the study. Dogs will be discharged on Day 26 (Friday) to their owners.
     3. After the Day 26 evaluation, dogs continue on their assigned schedule of rapamycin therapy. Monthly rechecks (Cycles 2, 3, and 4) are required as per study schema (Table IV).
     4. Subsequent study periods are defined as 28 days. The treatment interval will continue for up to 4 months in duration, as long as no severe adverse events are evident.
     5. Monthly rechecks on Day 25 of each respective 28-day dosing cycle (cycles 2, 3, and 4) are required prior to rapamycin administration. A CBC, chemistry profile, and UA will be collected and submitted in-house. Dogs should be fasted prior to presentation. A baseline whole blood sample (Time 0, trough) will be collected first, and then followed by rapamycin administration in-house by the COTC study coordinators. Whole blood will then be collected for a 6-point PK analysis over 24 hours. Dogs will be discharged on Day 26 (Friday) of each respective 28-day dosing cycle (cycles 2, 3, and 4) to their owners.
     6. Upon completion of PK analysis on Day 26 of cycle 2 and cycle 4, repeat 3-view thoracic radiographs will be completed.

**4.4 Study Schedule**

Pretreatment evaluations (week 15 of SOC protocol) will be performed within 7 days prior to initiation of rapamycin therapy and will include baseline examination, 3-view thoracic radiographs, and lab work. Rapamycin therapy will begin on a Monday (Day 1). Planned rapamycin treatment interval is 4 cycles (total of 16 weeks or 112 days). Rapamycin therapy will begin within a maximum of 7 days following Week 15 of the global study schedule.

#### Table II: Study Schedule Cycle 1

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Sunday** | **Monday** | **Tuesday** | **Wednesday** | **Thursday** | **Friday** | | **Saturday** |
|  |  | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | | **Day 6** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
|  | **Day 7** | **Day 8** | **Day 9** | **Day 10** | **Day 11** | **Day 12** | | **Day 13** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
| **PK collection** |  |  |  |  | multipoint 24 hr.  PK curve | | |  |
| **PE** |  |  |  |  | X |  | |  |
| **Serum, Whole Blood, PBMC** |  |  |  |  | X |  | |  |
|  | **Day 14** | **Day 15** | **Day 16** | **Day 17** | **Day 18** | **Day 19** | | **Day 20** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
|  | **Day 21** | **Day 22** | **Day 23** | **Day 24** | **Day 25** | **Day 26** | | **Day 27** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
| **PK collection** |  |  |  |  | multipoint 24 hr.  PK curve | | |  |
| **PE/labwork** |  |  |  |  | X | |  |  |
| **Serum, Whole Blood, PBMC** |  |  |  |  | X | |  |  |

**Table III: PK Whole Blood Collection Curve**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Time** | **0 (trough)** | **1 (hr)** | **2** | **4** | **6** | **8** | **24 hr** |
| Whole blood collection | X | X | X | X | X | X | X |

**Table IV: Cycle 2, 3 and 4 of Rapamycin therapy**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Sunday** | **Monday** | **Tuesday** | **Wednesday** | **Thursday** | **Friday** | | **Saturday** |
|  |  | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | | **Day 6** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
|  | **Day 7** | **Day 8** | **Day 9** | **Day 10** | **Day 11** | **Day 12** | | **Day 13** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
|  | **Day 14** | **Day 15** | **Day 16** | **Day 17** | **Day 18** | **Day 19** | | **Day 20** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
|  | **Day 21** | **Day 22** | **Day 23** | **Day 24** | **Day 25** | **Day 26** | | **Day 27** |
| **Rapamycin administration** |  | X | X | X | X |  | |  |
| **PK collection** |  |  |  |  | multipoint 24 hr.  PK curve1 | | |  |
| **PE&labwork1**  **Radiographs2** |  |  |  |  | X1 | | X2 |  |
| **Serum, whole blood, PBMC** |  |  |  |  | X | |  |  |

1Table III multipoint PK curve

2Repeat 3-view thoracic radiographs will be completed on Day 26 on Cycle 2 and Cycle 4

**4.5 Biological Collections and Application**

**Serum, whole blood, and PBMCs**

Serum will be collectedon Day 25 of each cycle. Serum collections will occur prior to Rapamycin administration. These collections will be maintained for post-hoc analysis.

Five serial whole blood collections for PK profiling will occur on study in dogs receiving rapamycin. Each PK profiling will include a pre-treatment sample (Time 0, trough) and additional 6-point serial whole blood collections over 24 hours. The pre-treatment sample (Time 0, trough) will serve as a standardized baseline for each patient. Times will include pre-treatment (Time 0, trough), 1 hr, 2, 4, 6, 8, and 24 hours. Serial whole blood collections will occur on Days 11 and 25 on cycle 1 and on Day 25 on cycles 2, 3 and 4.

PBMCs will be collectedon Day 25 of each cycle. PBMC collections will occur prior to Rapamycin administration. These collections will be maintained for post-hoc analysis.

**5.0 Clinical Evaluation:**

In this study, dogs are evaluated in the minimal residual disease setting after limb amputation. Therefore, the clinical and biological endpoints for study are restricted to the delay of clinically detectable pulmonary metastasis assessed with thorough physical examination and 3-view thoracic radiographs. In the event that a suspicious lesion(s) are detected via PE or imaging studies at any point during the study, confirmation of such lesion(s) as metastases may be necessary. Discussion with study investigators/DSMB may be required prior to deeming a dog off-study.

If progressive disease is detected or suspected based on suspicious lesions that develop during carboplatin chemotherapy or rapamycin therapy, repeat 3-view thoracic radiographs should be evaluated and compared to the previous radiographs in 4 weeks’ time. At that point, a decision regarding attribution of the suspicious lesion(s) will be made between the COTC clinician and Dr. Fan. At confirmation of progressive disease, dogs will be deemed off-study and may be offered alternative therapy.

**6.0 Toxicity**

Acute or chronic toxicity due to surgery for limb amputation, carboplatin and rapamycin exposures will be assessed within this trial design. Veterinary Cooperative Oncology Group Common Toxicity Criteria for Adverse Events (VCOG-CTCAE) will be used to determine dose-limiting toxicities (*Appendices II and III*).

**6.1 Drug Holidays-Rapamycin**

In the event that a dog experiences a Grade 2 toxicity for greater than 72 hours please contact Dr. Tim Fan to discuss. It is likely that a 7-day drug holiday will be implemented based upon the half-life of elimination of rapamycin, but ultimately will be based upon the time in course the events occur and upon case-specific discussions with the PI.There will be no dose reduction. All dogs will be treated with rapamycin at 0.1 mg/kg. After the 7-day drug holiday the dog will resume treatment at a modified schedule of treatment on Monday, Wednesday and Friday. **Recheck visits with collections will occur on a Friday instead of Thursday for dogs that are on the MWF schedule**. If a dog is unable to tolerate the modified MWF dosing schedule for 2 weeks or is still experiencing a Grade 2 event, the dog will be removed from study and is free to pursue alternative treatments.

**6.2 Study Stopping Rules**

In the event 33% of dogs require a drug holiday, the study schedule for all dogs will switch to Monday-Wednesday-Friday drug administration schedule. In the event 33% of dogs require a drug holiday on the MWF study schedule, the study would be temporarily suspended until the clinical data can be reviewed by the DSMB and a recommendation made for the ongoing conduct of the trial.

**7.0 Off Study Criteria**

Dogs will be removed from study if: a significant toxicity precluding further therapy occurs, ex post facto analysis reveals a patient did not meet eligibility criteria, owner requests withdrawal or disease progression is confirmed. The study period is 15 weeks for carboplatin administration and 16 weeks for rapamycin administration, with intervals of 8 weeks for clinical follow-up thereafter. The off study period begins on the day of confirmation of progressive disease.

**8.0 Study Communications**

Communications to the Principal investigators and Trial Coordinator can be done via text, email and phone call. The quickest and preferred form of communication is text. All emails for the study should include both Principal investigators and the Trial coordinator. Logistical questions should be directed to the Trial Coordinator. Eligibility questions can be directed to the Principal investigators with the Trial coordinator cc’d on all communications. Reporting for Adverse Events is detailed below in section 6.0.

1. Dr. Tim Fan: cell (217)-725-0573 t-fan@illinois.edu
2. Christina Mazcko: (240) 760-7094, cell (410) 562-9588

mazckoc@mail.nih.gov

**9.0 Adverse Events**

Adverse event data collection and reporting are required to ensure the safety of dogs enrolled on the study. This data will also contribute to the assessment of toxicity profile of this new agent. Adverse events will be reported in a routine manner at strictly enforced scheduled times during the trial, however certain adverse events must be reported in an expedited fashion to allow for possible study protocol modifications. **Adverse Reactions (AR)** are defined as any grade 1 toxicity. **Adverse Events** (AE) are defined as any expected or unexpected grade 2 or 3 toxicity, whereas a **Serious Adverse Event (SAE)** is any grade 4 or 5 toxicity expected or unexpected. It is essential that each adverse event be accurately graded based on severity and based on association with new drug exposure. All adverse events should be defined as unrelated, unlikely, possible, probable or definite(ly) associated with new agent (IND) exposure. All adverse events should also be defined as expected (based on this protocol) or unexpected.

* **Adverse Reaction Reporting:** an Adverse Event eCRF should be created within 48 hours of the event.
* **Adverse Event Reporting:** All Adverse Events should be reported within **24 hours** by emailing, texting or calling the Principal Investigator (Dr. Fan) or Trial Coordinator (Christina Mazcko). An Adverse Event eCRF should also be created within **24 hours** of the event. Principal Investigators or Trial Co-ordinator will notify study sponsors of all AE within 24 hours of initial reporting.
  + - * + Dr. Tim Fan: cell (217) 725-0573
        + Christina Mazcko: work (240) 760-7094, cell (410) 562-9588
* **Serious Adverse Event Reporting:** a Serious Adverse Event requires **immediate contact** by calling the Principal investigators. Also an Adverse Event eCRF should be created as soon as possible. It is expected that this contact should occur within 1 hour of any SAE. Principal Investigators or Trial Co-ordinator will notify study sponsors of all SAE within 24 hours of initial reporting.

Adverse effects are possible and the clinical scenarios of concern could include: vasculitis-gastrointestinal toxicity (diarrhea, anorexia, etc.), intussusception, pancreatitis, immunosuppression/infection, hypersensitivity, hyperlipidemia/hepatic dysfunction, thrombocytopenia, fever, pain, neutropenia, anemia, hyperglycemia, hypophosphotemia, rash, nephrotoxicity and death. **This list of clinical scenarios is not exhaustive, since this is a new use and formulation of this agent, hence actual individual case management will fall to the clinical judgment of the clinicians of record at the respective VTH and CCU.** There are study funds allocated for adverse event management related to rapamycin exposure only, two thousand dollars ($2000.00) per dog. This would fully account for all services provided in managing such events. The window for adverse event management includes all acute and chronic toxicities evident from the start of rapamycin therapy. If adverse events arise and unanticipated hospitalization is required then a 48-72 hour treatment period would be appropriate to determine if a dog is to likely recover from these events. Owners will be kept fully informed of any adverse events and their subsequent management. **Also, communication with the Principal Investigators or Trial Coordinator is required on any cases receiving care for AE or SAE.** If serious adverse events occur, owners can elect euthanasia, however a necropsy is then required (*Appendix I. SOP 05)*. Dogs who experience adverse events may be delayed from subsequent rapamycin administration or modification in schedule or dose may be necessary before re-initiation. A discussion between the Principal investigators and the COTC member investigators would determine if such a protocol delay or dose modification is to occur. Some suggested guidelines for management of a patient experiencing an adverse event are defined below, again however, clinicians are at liberty to treat adverse events as clinically indicated.

**Adverse Events Clinical Management Guidelines:**

**THE FIRST CLINICAL MANAGEMENT REQUIREMENT FOR ALL DOGS PRESENTING WITH AN ADVERSE EVENT IS TO DRAW A WHOLE BLOOD SAMPLE FOR PK ANALYSIS *(SOP02).* THIS IS REGARDLESS OF TIME IN COURSE. THIS SAMPLE SHOULD BE LABELLED WITH DATE AND TIME OF COLLECTION. THEN ADDED TO eCRF.**

1. **Gastrointestinal toxicity**: defined as vomiting or diarrhea.

a. **Cease rapamycin dosing until contact with COP PIs and collect whole blood for PK**

b. Vomiting-make patient NPO. If vomiting persists administer Metoclopramide 0.4 mg/kg SQ or CRI or Cerenia 1 mg/kg SQ. If vomiting is intractable Zofran/Anzamet may be added IV/PO.

c. Diarrhea- make patient NPO. Add a bland diet. Prescribe Metronidazole 10-15 mg/kg IV/PO.

d. If either is protracted or severe initiate IV fluids-crystalloids at 60-90 ml/kg/hr.

e. **RULE OUT INTUSSUSCEPTION**

f. Assess CBC and biochemical profile.

2. **Pancreatitis**: defined by clinical signs of abdominal pain, vomiting, anorexia, elevated amylase, lipase

a. Assess biochemical profile

b. Initiate IV fluids-crystalloids at 60-90 ml/kg/hr and if necessary colloids at

5-10 ml/kg.

c. Treat vomiting as above

d. Administer plasma transfusion

3. **Hypersensitivity/allergic reaction**: defined as wheal formation, vomiting, hypotension and/or shock. If hypersensitivity occurs following oral rapamycin administration it should be terminated and the PIs contacted prior to further administration. In the acute situation:

a. Initiate shock fluid therapy at 90 ml/kg IV.

b. Administer Benadryl 1 mg/kg IM and Dexamethasone 0.5-1 mg/kg IV.

c. If clinical status does not improve, administer epinephrine.

4. **Fever/Infection secondary to immunosuppression**: defined as >104 F with lethargy

a. Assess CBC and culture urine/blood if indicated

b. Initiate IV fluids-crystalloids at 60-90 ml/kg/hr

c. If T > 106 F or is persistent, administer NSAIDs or Tylenol

5. **Thrombocytopenia**: platelets < 50,000

a. Cease rapamycin dosing

b. Assess coagulation (PT, PTT)

c. Administer whole blood or platelet rich plasma if clinician deems indicated

6. **Hyperlipidemia/Hepatic dysfunction**: defined as elevations in cholesterol, alkaline phosphatase, ALT, increased bile acids, icterus-that is clinically relevant

a. Assess biochemical profile and coagulation (PT, PTT)

b. Treat vomiting, diarrhea or other clinical signs

c. Administer plasma transfusion and reassess coagulation parameters upon completion. This can be repeated if clinically indicated and there has been some initial improvement of clotting parameters.

7. **Nephrotoxicity**: defined by elevations in BUN/Cr with isosthuria above baseline

a. **Cease rapamycin dosing until contact with COP PIs**

b. Initiate IV fluids-crystalloids at 60-90 ml/kg/hr if indicated

c. Treat associated clinical signs: vomiting, nausea, anorexia, etc with appropriate

symptomatic therapy: Metoclopramide, Famotidine, Aluminum Hydroxide, etc.

The principal investigators should be kept abreast of the clinical status of any patients that undergo adverse reactions. The Principal Investigators are available to discuss the clinical management of individual study patients as needed, please call with questions if they arise.

**9.1 Reconciliation and Attribution of Adverse Events and Severe Adverse Events**

Initial reporting and coding of Adverse Events and Severe Adverse Events should be completed according to the timeline outlined in section 5.0. Reconciliation of AE and SAE are required at two time points. The first is 7 days after initial reporting of an AE or SAE and the second within 7 days of a dog completing the study or being removed from the study. Reconciliation should be based on all available clinical data related to study patient management. On a weekly basis the Trial Coordinator will conduct verification of adverse event reporting. Reconciliation of AE and SAE requires discussion between COTC investigators and Principal Investigators. This discussion will be initiated and coordinated by the Trial Coordinator. Reconciliation involves re-examination of each AE or SAE in terms of severity classification, association with new agent exposure, and whether the event is expected or unexpected. It is during this reconciliation process that attributions will be assigned to each Adverse Event. Attribution to research protocol, rapamycin (IND), disease or other will be assigned and agreed upon by the COTC investigator and Principal Investigators. Attribution is defined as the following (unrelated, unlikely, possible, probably, definite).

**10.0 Necropsy**

Necropsy is required for any dog that dies while on study.Necropsy is encouraged in dogs that complete the study but die at a later date without having received any further therapies. Necropsy is not required in dogs that go off study or those who receive other treatment regimes after study completion. At death, a full necropsy (*SOP 05)* should be performed in an expedient fashion and abnormal tissues submitted for standard histopathologic analysis at the COTC member site.

**11.0 Data Reporting and Record Keeping**

**Study Standards:** These studies are pilot studies of the safety and efficacy of oral rapamycin therapy in tumor bearing dogs. Where practical, Good Clinical Practice standards will be followed.

**11.1 Reporting**: All data will be collected and stored in C3D, an electronic clinical database. Data must be contemporaneous and submitted to C3D weekly. Initial screening eCRFs must be completed prior the first rapamycin treatment. All adverse event reporting should be conducted as described above. Each study day and its corresponding eCRFs are listed below. The location of each eCRF within C3D is also noted. eCRF completion is required within **one week of a scheduled visit**. Non-compliance with eCRF completion will limit further patient accrual. All remaining patient eCRFs should be completed within one week from a patient being off study. Complete records should be maintained on each patient within their individual COTC site VTH as per standard record keeping methods. Any unanticipated or unknown treatment related toxicities will be reported in accordance to guidelines prescribed by the COP. All COTC participating institutions will be made aware of adverse reactions that occur in other study sites. Data amassed during the study period may not be disclosed to non-study participants without the written consent of the Principal investigators.

**11.2 Verification of C3D Data Entry**: The COP Study Coordinator and Principal Investigators oversee all clinical data entered into C3D. The Study Coordinator on a weekly basis performs verification of C3D data entry. Verification is aimed to ensure data is captured accurately and fully according to protocol guidelines, entered contemporaneously and that adverse event reporting is reconciled as directed in section 5.1.

**12.0 Study Drug Formulation**

Rapamycin will be provided as tablets (0.5 mg and 2.0 mg) from NCI-COP with appropriate amounts for each treatment cycle (Cycles 1-4), and will be dispensed for each patient based upon body weight.

**13.0 Study Drug Management**

**13.1 Drug Storage**: Study drugs are to be stored in a secured and locked location at room temperature (20-25 C) and protected from light. Neither the investigator nor any of their designees may provide study drug to any dog not participating in this protocol.

**13.2 Drug Administration**: Study medications will be dispensed to individual dogs at the dose prescribed being 0.1 mg/kg Monday-Thursday. In the event of rapamycin intolerability, a dose reduction will be instituted, with rapamycin dosed at 0.1 mg/kg M,W,F scheme. Rapamycin will be administered to patients in the morning following a 4-hour fast and no sooner than 1-hour prior to feeding.

Owners should be instructed to monitor pets to ensure drug administration during meals. Owners will be required to document their daily administration of rapamycin on a client administration chart (Appendix I. 4.0). The owner must return the **Client Rapamycin Administration Form** to the hospital on scheduled reevaluations, being Cycle 1 on Days 11 and Day 25 and Cycles 2-4 on Day 25.

**13.3 Drug Reconciliation and Dose Verification:** All unused drug must be returned to the COTC institution where drug reconciliation will be performed weekly. Any unused rapamycin should be disposed of following standard drug disposal guidelines. Institutions should notify NCI with any variance from expected to actual dose. Enter data into Study Medication eCRF.

**13.4 Human Contact:** While administering rapamycin to patients, clinicians/nurses and owners should wear plastic, chemotherapy gloves. Pregnant women or those individuals who are immunosuppressed should not handle rapamycin. If a client has concerns about their immune status, they should consult their physician prior to enrolling their dog in this clinical trial.

**14.0 Future Use of Collected Data/Samples:**

**14.1 Biological endpoints to be assessed:**

* Rapamycin pharmacokinetics via whole blood rapamycin levels