A PILOT STUDY EVALUATING THE USE OF THE mTOR INHIBITOR SIROLIMUS IN CHILDREN AND YOUNG ADULTS WITH DESMOID-TYPE FIBROMATOSIS.

Co-Principal Investigators:

Aaron Weiss, DO
Maine Children's Cancer Program
100 Campus Drive, Unit 107
Scarborough, ME 04074
Phone:207.396.7565 Fax: 207.396.7577
Email : weissa2@mmc.org

Noah Federman, MD
David Geffen School of Medicine at UCLA
Pediatric Hematology/Oncology, MDCC A2-410,
10833 Le Conte Avenue, Los Angeles, CA
90095
Phone: 310.825.6708 Fax: 310.206.8089
Email: nfederman@mednet.ucla.edu

Research Coordinator:

Kathleen M. Glick, CCRP
Coordinator of Clinical Research
Maine Children's Cancer Program
100 Campus Drive
Scarborough, ME 04074
Phone: 207.396.7565 Fax: 207.396.7577
Email: glickk@mmc.org

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OVERVIEW

Desmoid-type fibromatosis (or desmoid tumor) represents an intermediate grade neoplasm with a striking predilection for locally invasive growth and recurrence following resection. It occurs in children as well as young adults. As a typically localized disease, the historical standard of care for treatment has been surgical resection, with or without ionizing radiation. In some cases where surgical resection or radiation is not feasible, chemotherapy has been used. Two clinical trials conducted in the Pediatric Oncology Group (POG) and the Children's Oncology Group (COG) evaluated the role for either low intensity or non-cytotoxic chemotherapy for children with desmoid tumor that is not amenable to standard therapy. These were largely empirical treatment strategies or based on somewhat anecdotal observations. By better understanding desmoid tumor biology, even more effective therapy targeting a particular protein that is central to the disease can be developed.

Desmoid tumor is well-known to be associated with deregulation of the APC/β-catenin pathway. This is true of familial cases associated with Gardner's Syndrome and also in sporadic desmoid tumor, nearly all of which display histological or molecular evidence of APC/β-catenin pathway activation (Alman et al., 1997; Lips et al., 2009). Several new pieces of evidence support the concept that deregulation of the mTOR cell proliferation/survival pathway may play an important role in tumor biology when the APC/β-catenin pathway is disrupted. Sirolimus, a drug that inhibits mTOR, is currently being evaluated as an anti-cancer agent in a variety of tumor types, but it has not been previously studied in desmoid tumor.

We are conducting this pilot study to begin to explore whether mTOR inhibition may be beneficial for children and young adults with desmoid tumor.

OBJECTIVES

Primary

1. To determine whether mTOR pathway activation decreases in patients with surgically-resectable desmoid tumor that is removed following pre-operative treatment with sirolimus.

Secondary

1. To assess whether sirolimus improves desmoid tumor-associated pain.
2. To begin to explore whether pre-operative sirolimus decreases tumor recurrence following surgical removal of desmoid tumor felt to be at high-risk for recurrence because of size and/or anatomic site.
3. To assess the safety and tolerability of pre-operative sirolimus in patients with desmoid tumor.

BACKGROUND

Past experience using chemotherapy for desmoid tumor

Effective treatment of desmoid-type fibromatosis (desmoid tumor) poses two major clinical problems. One major problem is how to best control disease that cannot be resected or treated with radiation, both of which represent the historical standards of care for adults with desmoid tumor. Interestingly, though, radiation therapy may be less effective for children with this

disease (Merchant et al., 2000). Further, the high doses of radiation that are required can make its use problematic for disease at certain anatomic sites and in children.

A second problem relates to preventing disease recurrence in those patients with resectable disease. Recurrence rates are highly variable and depend largely on whether the tumor has been completely excised. For example, Faulkner and colleagues show a 75% recurrence rate in a series of 63 pediatric patients (Faulkner et al., 1995); however, recurrence-free survival after three years approximates 70% and 10% in patients with negative margins and positive margins, respectively. Some pre-operative predictors of recurrence risk have been proposed. For example, two retrospective series indicate that chance of getting a complete resection is smaller in those with tumor > 5 cm (Rao et al., 1987; Meazza et al., 2010); however, tumor size alone was not predictive of outcome in other series (Faulkner et al., 1995; Merchant et al., 1999). Ballo and colleagues found that, in addition to positive surgical margins, age $\leq$ 30 years was associated with significantly more relapse (Ballo et al., 1999); recurrence tended to be greater in those with extremity desmoid tumor and those with more than one prior treatment, but this did not reach statistical significance. Post-operative radiation decreases the recurrence risk (Ballo et al., 1999) but, as noted above, its use may be constrained by certain anatomic sites, especially in children where skeletal and muscle growth and development are added concerns.

Some effort has been devoted to attempting to use systemic chemotherapy to ameliorate these problems. Much of what is known about chemotherapy in patients with desmoid-type fibromatosis stems from small, single institution, retrospective analyses. Cytotoxic agents demonstrated to have some activity include vincristine/actinomycin/cyclophosphamide (Raney et al., 1987), liposomal doxorubicin (Wehl et al., 2004), continuous infusion doxorubicin/DTIC (with or without meloxicam) (Constantinidou et al., 2009; Gega et al., 2006), hydroxyurea (personal communication, R. Womer, U. Penn), and vinblastine/methotrexate (Skapek et al., 2007; Azzarelli et al., 2001; Weiss and Lackman, 1989). Response rates range from 36-80%. “Targeted drugs” such as imatinib (Heinrich et al., 2006; Mace et al., 2002) and sunitinib (Skubitz et al., 2009), have also been used in small series. Lastly, “non-cytotoxic” agents with some activity include tamoxifen (with or without a variety of non-steroidal anti-inflammatory drugs) (Hansmann et al., 2003) and non-steroidal anti-inflammatory drugs as single agents(Klein et al., 1987) (Janinis et al., 2003).

In an effort to better understand chemotherapy effectiveness for children with desmoid-type fibromatosis, two consecutive, prospective, multi-institutional clinical trials were completed in children with desmoid tumor that was either recurrent or not amenable to surgical resection or radiation. The first, conducted within the Pediatric Oncology Group (POG Study 9650), investigated the efficacy and safety of vinblastine and methotrexate (Skapek et al., 2007). Benefit was evidenced by disease stabilization or regression in approximately two thirds of children enrolled on the study. The 1 year progression-free survival was 58%. Toxicity was significant with 66% of subjects experiencing grade 3 or 4 toxicity. The second study, conducted within the Children’s Oncology Group (COG study ARST0321) tested the efficacy of high-dose tamoxifen and sulindac in a similar population. This study completed accrual in May 2009; a preliminary report from the COG Data Safety and Monitoring Committee indicates that the failure-free survival with tamoxifen and sulindac will not be better that that achieved with vinblastine and methotrexate (unpublished data from COG Statistics and Data Center). Toxicities, although different from POG9650, were not insignificant.

Given the less-than-ideal success of these chemotherapy approaches, there is a need to evaluate other drugs that may be more effective and better tolerated. The current pilot study differs from these prior chemotherapy studies in children because it is focused on using pre-operative chemotherapy. This will offer a window to judge chemotherapy efficacy, based on improved symptoms of pain which commonly precede objective decreases in tumor size [for example, (Weiss and Lackman, 1989)]. Conceptually, pre-operative chemotherapy may decrease the chance for tumor recurrence following surgery and, if there is evidence for efficacy, it can be continued following surgery. Beyond this potential benefit, the primary objective is to gain evidence of response to sirolimus by using well-described, histochemical studies that correlate with blockade of the mTOR pathway by sirolimus and similar drugs. The strategy will provide concrete evidence as to whether the drug effectively "hits" its target in children and young adults with the disease following a relatively short treatment.

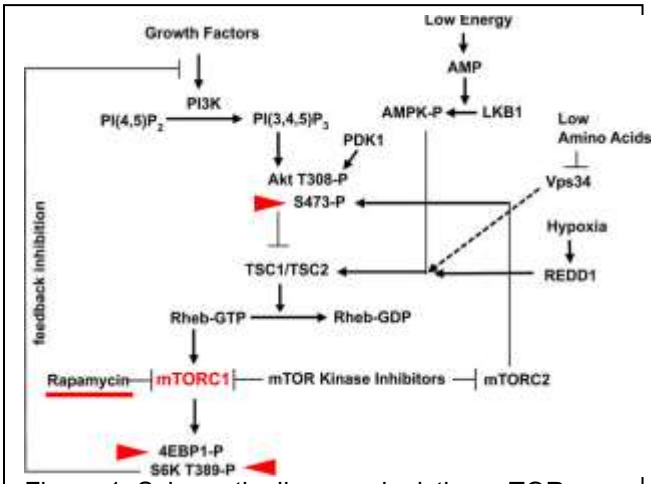

Figure 1: Schematic diagram depicting mTOR pathway. Arrows denote proteins that will be evaluated as part of this study (see below).

mTOR as a potential therapeutic target

Several independent lines of evidence suggest that the mTOR kinase may represent an important therapeutic target in this disease. mTOR forms two separate complexes with additional proteins to influence a variety of cellular pathways (Ballou and Lin, 2008). First, it physically interacts with RAPTOR to form the mTORC1 complex, which fosters cell growth, proliferation, motility, and angiogenesis. mTOR also interacts with RICTOR to form the mTORC2 complex; TORC2 phosphorylates AKT to control cytoskeleton organization (Figure 1). The mTORC1 complex appears to be the most critical pathway for tumorigenesis. The mTOR kinase has been established to have a range of tumorigenic effects in human cancer (Bjornsti and Houghton, 2004).

Support for the role that mTOR may play in desmoid tumor comes, in part, from pre-clinical studies in a mouse model of Gardner's Syndrome/FAP (Fujishita et al., 2008). This is potentially relevant because nearly all desmoid tumor specimens show evidence for *APC* gene mutation or deregulation of its down-stream effector, β-catenin (Alman et al., 1997; Lips et al., 2009; Miyoshi et al., 1998). Mice bearing a germ-line mutant $Apc^{\Delta 716}$ allele develop intestinal polyps (Oshima et al., 1995). It was recently shown that mTOR expression and mTORC1 activity are increased in these intestinal polyps (Fujishita et al., 2008). The functional importance of mTORC1 comes from the finding that RAD001 (everolimus), an mTOR inhibitor, (a) suppresses mTORC1 expression, (b) inhibits adenoma cell proliferation, (c) decreases the number and size of intestinal polyps, (d) impedes tumor angiogenesis, and (e) prolongs animal survival. The treatment effects were limited to the polyps and vascular epithelial cells while sparing the adjacent normal intestinal mucosa. Further, knockdown of β-catenin using siRNA similarly decreased mTOR expression (Fujishita et al., 2008).

Numerous studies have used immunohistochemical (IHC) staining to assess mTOR pathway activation with phospho-specific antibodies against AKT, S6K, and eIF4E-BP1 [for example, (Chung et al., 2009; Baba et al., 2009)]. To our knowledge, this has not been addressed in desmoid tumor. We have explored this using a single archived desmoid tumor specimen, and showed that both AKT and S6 kinase are phosphorylated in the tumor cells (Figure 2).

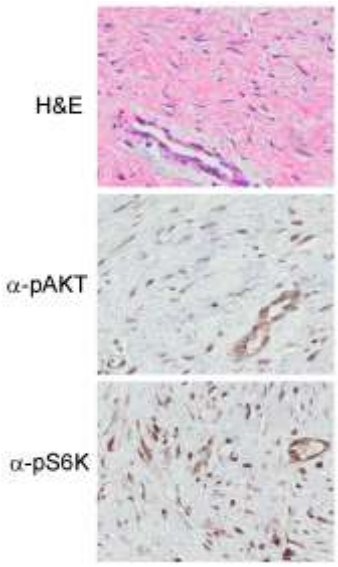

Figure 2: Representative IHC staining for indicated proteins in a desmoid tumor. No staining observed in negative control done in parallel (negative data not shown).

Lastly, a recent case report provides what appears to be the first published evidence of mTOR inhibition in desmoid tumor. In this case, a 7 year old male with tuberous sclerosis and a recurrent chest wall desmoid tumor was treated using sirolimus (Pressey et al., 2010). The tumor size decreased from 5.63 to 4.05 cm and developed septations suggestive of tumor necrosis. The response was maintained for 22 months.

These preliminary pre-clinical, translational, and clinical findings support the notion that mTOR may control tumorigenic aspects of desmoid tumor and represent a rational target.

Previous clinical experience with sirolimus and related drugs in cancer

Three mTOR inhibitors are available for use in the clinic: sirolimus (Rapamune®, Rapamycin), temsirolimus (Torisel®, CCI-779), and everolimus (Afinitor®, RAD001). mTOR inhibition in cancer has included a number of phase I and phase II studies. Anti-tumor activity of mTOR inhibitors has been observed in adult patients in a range of cancers [for example, (Witzig et al., 2005; Amato et al., 2008; Mita et al., 2008)]. Pre-clinical findings and results emerging from clinical trials supports the use of mTOR inhibitors in sarcoma as well (Wan and Helman, 2007). This may be particularly relevant because desmoid tumors are of mesenchymal origin and share biological properties with sarcoma subtypes.

For the most part, mTOR inhibitors have tolerable side effects: The sarcoma study by Chawla and colleagues revealed minimal Grade 3 or 4 adverse drug reactions. The most common toxicities were Grade 1 and 2 toxicities: mucositis, rash, hypertriglyceridemia, and fatigue. Everolimus was studied in a group of 92 patients with advanced solid tumors (O'Donnell et al., 2008). Partial responses were seen in 4 patients while 12 patients experienced prolonged disease stabilization. The most common non-hematologic adverse drug events involved mucositis, rash, and fatigue. Grade 3 and 4 toxicities were infrequent.

Because mTOR inhibitors are thought to act, in part, by interfering with angiogenesis, there is a theoretical concern that they may also interfere with surgical wound healing. While there are no published data about the use of sirolimus in this specific setting, there are limited publications discussing the risk of sirolimus in the peri-operative time frame in other disease types (Kuppahally et al., 2006; Tiong et al., 2009; Goldberg et al., 2014; Schwarz et al., 2014; Heble et al., 2018). In most of these studies sirolimus was used de novo following surgery. Although the incidence of surgical wound complications was low in the few studies reviewing the risk when sirolimus was used pre-operatively (Schwarz et al., 2014; Heble et al., 2018), as in this study, we will carefully monitor surgical complications and wound healing and terminate the study, should a significant number of surgical complications be observed.

Preclinical use of sirolimus in pediatric cancer

Initial testing of sirolimus by the Pediatric Preclinical Testing Program demonstrated broad antitumor activity. Sirolimus induced 50% or greater growth inhibition in 10 of 23 cell lines (Houghton et al., 2008). Against in vivo panels, sirolimus induced significant differences in event free survival in 27 of 36 (75%) solid tumor xenografts when compared to controls. Tumor regression was observed in several solid tumor models, most notably, rhabdoid tumor, rhabdomyosarcoma, and osteosarcoma.

mTOR inhibitor use in children

Each of the above-mentioned mTOR inhibitors has completed Phase I testing in children.

A Phase I study of everolimus in pediatric patients with refractory solid tumors demonstrated prolonged disease stabilization in patients with osteosarcoma and peripheral PNET (Fouladi et al., 2007). Everolimus was found to be well tolerated in children with dose limiting toxicity associated with diarrhea (n=1), mucositis (n=1), and elevation in ALT (n=1).

Sirolimus has been routinely used as an immunosuppressive drug after solid organ transplant and to control graft-versus-host disease following hematopoietic stem cell transplant in children (Campistol et al., 2009; Buhaescu et al., 2006). In these contexts, doses range from 2 to 5 mg/m^2/day, which result in trough levels from 5 to 20 mg/mL.

The standard sirolimus dose in adults is a 12 mg PO loading dose and 4 mg PO daily. Sirolimus has also been used in a Phase I study conducted at The Children's Hospital of Philadelphia in pediatric patients with acute leukemia in the second or subsequent relapse (personal communication: S. Rheingold, Children's Hospital of Philadelphia and 2007 American Society of Hematology abstract). No dose-limiting toxicities were observed at the first two dose levels (loading dose: 9 mg/m^2, subsequent daily dose: 3 mg/m^2; and loading dose: 12 mg/m^2, subsequent daily dose: 4 mg/m^2). Trough levels ranged from 7 to 9 and 10 to 11 ng/ml at the first and second dose level, respectively.

Because obvious differences in efficacy of the three available mTOR inhibitors do not exist (Wan and Helman, 2007), sirolimus will be used in this study.

Pain as a Surrogate Marker of Response in Desmoid Tumor

Desmoid tumor represents a heterogeneous disease and can have an unpredictable clinical course, particular related to growth rate (De, I et al., 2000; de et al., 2009). Often, the slow growth and slow response of desmoid tumor precludes the use of previously established standardized radiographic measurements of response, such as the Response Evaluation Criteria in Solid Tumors (RECIST). This is particularly true in shorter treatment schedules such as the one proposed in this pilot study. Therefore, other surrogate markers of response are needed.

Burris and colleagues were one of the first groups to look at pain as a measure of 'clinical benefit' within the context of a therapeutic cancer study (Burris, III et al., 1997). Clinical benefit was a composite assessment of pain, performance status, and weight. Clinical benefit was measured prospectively as a primary endpoint of the study. Patients with advanced pancreatic cancer were randomized to receive gemcitabine or 5-fluorouracil (5-FU). The designation for pain integrated both the subjective report of pain intensity as well as analgesic consumption.

Pain intensity was measured daily on the Memorial Pain Assessment Card (MPAC) 0-100 visual analog scale, and a positive response was associated with an improvement of ≥ 50% from baseline sustained for ≥ 4 weeks. Analgesic consumption was measured weekly in morphine-equivalent milligrams, and a positive response was indicated by a decrease of ≥ 50% from baseline, sustained for ≥ 4 weeks). Clinical benefit was experienced by 24% of gemcitabine-treated patients compared with 5% of 5-FU treated patients.

Recently, Ohorodnyk and co-authors performed a literature search for reports of all clinical trials (phase I, II, and III) published in the Journal of Clinical Oncology from 1997-2008 citing 'clinical benefit' (Ohorodnyk et al., 2009). Eligible trials were those reporting clinical benefit as an endpoint. Clinical benefit was classified as patient-centered if it referred to improvement in the clinical parameters used by Burris and colleagues or in other disease-related symptoms. 71 trials reporting clinical benefit were identified: 37 in breast, 8 in pancreas and 26 in other cancers. Among the 71 trials reporting clinical benefit, 31 (44%) trials had that endpoint defined as a primary or secondary study objective. Of note, the authors found a steady increase in the number of trials using clinical benefit as an endpoint; in the second half of the study period the number of trials increased from 17 to 54.

The locally invasive nature of desmoid tumor frequently results in significant pain (Hosalkar et al., 2006). Improvement in pain for patients undergoing treatment for desmoid tumor has been described in a few previous studies. Constantinidou et al demonstrated improvement in pain with the use of pegylated liposome doxorubicin (PLD) (Constantinidou et al., 2009). Eleven of 12 patients with desmoid tumor treated with PLD had clinical benefit in terms of pain relief or improved mobility and cosmesis. Skubitz and colleagues noted improved pain in a sunitinib-treated patient with recurrent desmoid tumor (Skubitz et al., 2009). The pain improved and resolved by 28 days of treatment. A reduction in tumor size was noted after 5 months of therapy. Neither study mentioned the specific pain assessment tool utilized.

To our knowledge, no pediatric study has used pain as a primary or secondary endpoint in cancer treatment. Because of pain often associated with desmoid tumor and the slow changes in tumor size, we will use pain assessment as a surrogate marker of response in this study. Validated pain assessment scales, depending on the age of the patient, have been established for our patient population (Bijur et al., 2003; Hain, 1997; von Baeyer, 2006). For this pilot study, the Wong-Baker FACES scale will be used for patients ≥ 3 and < 10 years of age and the numeric scale will be used for patients who are 10 years of age or older.

Summary of trial design

Our overall goal is to conduct a pilot study to begin to assess whether a four week course of sirolimus, given prior to planned surgery, can decrease evidence of mTOR activity, decrease tumor associated pain, and decrease tumor recurrence. The target patient population will be patients < age 30 years at the time of original diagnosis in whom surgery is planned to remove the desmoid tumor and either (a) the desmoid tumor has already recurred after a prior surgery or (b) the newly diagnosed and/or previously unresected disease is judged to be at high risk for recurrence due to its size (> 5cm) or location at an anatomic site making it unlikely to be resected with negative margins (*e.g.*, adjacent to neurovascular structures).

A total of 15 subjects will be enrolled in the trial. The primary endpoint will be achieved by performing immunohistochemical studies for mTOR pathway activation on the resected tumor samples. Immunostaining results will be compared to a cohort of previously untreated,

archived specimens (n=50), and also by paired comparison with pre-treatment tumor or biopsy specimens if available.

The secondary endpoints will be accomplished using validated pain assessment scales at weekly intervals and routine, post-operative surveillance imaging.
This clinical trial is an IND exempt study. Maine Medical Center (MMC)/Maine Children's Cancer Program (MCCP) will be the coordinating center for this multi-site trial. As the coordinating center we will ensure that IRB documentation is in place at each of the participating sites. Participating sites will submit to Dr. Weiss at MMC/MCCP local reportable adverse events, protocol deviations, and any reportable new information as well as require each local site to submit this to their IRB per local policy

SUBJECT RECRUITMENT

Subjects will be identified upon diagnosis by attending orthopedic surgeons, general surgeons or oncology physicians at the participating enrolling institution. Disease evaluation and routine tests will take place at these sites. In some instances, laboratory tests may be collected either by local clinics and results will be faxed to the attending physician. Subjects will not be paid for participation on this protocol. In addition, all tests and study drugs that are considered standard of care will be charged to the patient or their insurer.

ELIGIBILITY

Inclusion criteria

- Must be less than 30 years of age at the time of original diagnosis.
- Must have biopsy-proven desmoid tumor (or aggressive fibromatosis). For patients with recurrent disease, a biopsy is not required at the time of recurrence.
- Patients known to have germ-line APC mutations or clinical manifestations of FAP/Gardner's syndrome can be included.
- Patients must have surgery planned to remove the desmoid tumor and either:
    a) The desmoid tumor has already recurred after a prior surgery or
    b) The newly diagnosed and/or previously unresected disease is judged to be at high risk for recurrence due to its size (> 5 cm) or location at an anatomic site making it unlikely to be resected with negative margins (*e.g.*, adjacent to neurovascular structures)
- There must be a commitment by the surgical team to resect the primary tumor within 3 days following the 4 weeks of sirolimus unless the clinical situation at the time of resection suggests that these interventions are not in the patient's best interest.
- Concomitant medication restrictions:
    a) Patients may have received prior chemotherapy (excluding prior mTOR inhibitors).
    b) Use of steroids for non-tumor indications (e.g., asthma or severe allergic reaction) is permitted.
- Patients must have a Karnofsky performance status ≥ 50 for patients > 16 years of age or Lansky performance status ≥ 50 for patients ≤16 years of age.
- Patients must have a life expectancy ≥ 8 weeks.
- Patients must have recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.
    a) Myelosuppressive chemotherapy: Must not have received within 2 weeks of entry onto this study (4 weeks if prior nitrosourea).

b) Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with a biological agent.
c) Stem Cell Transplant (SCT): No evidence of active graft vs. host disease. For allogeneic SCT, ≥ 6 months must have elapsed.

- Patients must be able to consume oral medication in the form of tablets or solution.
- Patients must have normal laboratory values as defined below:
  - Creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73m^2 or
    - - A serum creatinine based on age/gender as follows:

Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

- Hepatic
  - Adequate liver function defined as:
    - Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and
    - SGPT (ALT) ≤ 2.5 x upper limit of normal (ULN) for age.

- Hematologic function
  - Adequate bone marrow function defined as:
    - ANC ≥ 1 × 10^9/L
    - Hemoglobin ≥ 10g/dL
    - Platelet count ≥ 100 × 10^9/L

- Female patients must have a negative pregnancy test.
- Female patients who are lactating must agree to stop breast-feeding.
- Sexually active patients of childbearing potential must agree to use effective contraception.
- Patients must be able to cooperate fully with all planned protocol therapy.
- Signed informed consent MUST be obtained from patient or parent/legal guardian (if patient is <18 yrs of age). Consent must be obtained prior to any study procedures and study entry.

Exclusion criteria

- Patients with other fibroblastic lesions or other fibromatoses are NOT eligible.
- Concomitant medications restrictions:
  a) Patients may NOT have received prior mTOR inhibitors.
  b) Growth factor(s): Must not have received within 1 week of entry onto this study.

- Patients must not be known to be HIV positive. Testing for HIV is not mandatory.
- Patients must not be taking medicines known to influence sirolimus metabolism (see Table on Page 15).

Regulatory

- All patients and/or their parents or legal guardians must sign a written informed consent.
- All institutional, FDA, and NCI requirements for human studies must be met.
- As the coordinating center MMC/MCCP will obtain a copy of each local sites IRB approval and IRB approved informed consent documents and all subsequent IRB reportable changes.

REGISTRATION PROCEDURES

All eligible subjects that provide consent to this study will be registered at MMC/MCCP. Eligibility will be verified with the Investigator or treating physician prior to registering the subject to study.

REQUIRED OBSERVATIONS

Tumor evaluation must be completed within 21 days prior to study entry. In addition, labs and physical exam must be completed within 7 days prior to study entry. Therapy must begin within 21 days following signing informed consent document.

Other recommended and required observations are detailed in the following Tables:

Evaluations	Prior to Study Entry*	During Course 1**	Prior to Surgery*
History	X	Weekly	
Physical Exam (HT, WT, BSA, VS)	X	Weekly	X
Performance Status	X		
Pain Scale[1]	X	Weekly (required)	X
CBC, differential, platelets	X	Weekly	X
Serum electrolytes including Ca++, PO4, Mg++	X	Weekly	X
Creatinine, SGPT, bilirubin	X	Weekly	X
Fasting Glucose/Cholesterol/Triglyceride	X	Prior to week 3	X
Urine Pregancy Test[2]	X		
Urinalysis	X		
Trough Sirolimus Level		Weekly	
MRI of Primary[3]	X		X[4]

* All evaluations required except where noted.
** Course 1 represents the first 4 weeks of sirolimus; all evaluations strongly recommended except where otherwise noted.
[1] Patients ≥3 and < 10 years of age will use the Wong-Baker FACES scale; patients who are ≥ 10 years of age will use the numeric scale. See Appendix 1.
[2] Patients of childbearing potential require a negative pregnancy test prior to starting treatment.
[3] MRI should include images in at least two planes with (a) pre-contrast images with the following pulse sequences (1) T-1 weighted, (2) fast spin echo T-2 weighted with fat saturation OR a short tau inversion recovery (STIR); and (b) post-contrast images with T-1 weighted pulse sequence with fat suppression.
[4] Optional but strongly recommended. If performed, attempt to obtain as close to planned surgery as possible.

Post-surgery follow-up evaluation – months post therapy

Evaluations post-Surgery*	**3**	**6**	**9**	**12**	**18**	**24**	**36**
History	x	x	x	x	x	x	x
Physical Exam (HT, WT, BSA, VS)	x	x	x	x	x	x	x
MRI of Primary	x	x	x	x	x	x	x
Data Collection Forms[1] (required)	x	x	x	x	x	x	x
Operative Report (required)	x						

* All evaluations strongly recommended except where otherwise noted.
[1]See Case Report Forms

TREATMENT PLAN

Preoperative Chemotherapy for all patients

- Sirolimus:
  - Loading dose of 12 mg/m^2; PO day 1 **(MAX dose 12 mg)**
  - Starting 24 hours after the initial loading dose, patients will receive a dose of 4 mg/m^2 daily; PO days 2 through 28 **(MAX dose 4 mg/day)**

If the patient suffers any Grade 3 or 4 toxicity that the treating physician feels may delay surgery, the patient will come off study.

Surgical Resection for all patients

- Sirolimus will be discontinued following 28 days of therapy. Surgical resection should be accomplished within 3 days of completing therapy. The tumor will be processed for routine histology as well as for study specific tests.
- Anticipated or unanticipated surgical delay beyond 3 days should be discussed with the study PI to determine if a patient should be removed from protocol therapy.

Note about Postoperative Chemotherapy

- *The pilot study is complete following tumor resection. If the treating physician chooses to continue sirolimus following resection, for example, based on symptomatic improvement, it will be independent of this clinical trial.*

DRUG ADMINISTRATION GUIDELINES:

- **Sirolimus**
  - Prior to beginning and each week, the following laboratory criteria must be met before beginning or continuing Sirolimus:
    - ANC $\geq$ 0.75 x 10^9/L
    - Platelets $\geq$ 75 x 10^9/L
    - Serum creatinine $\leq$ 2 x baseline or GFR $\geq$ 70 mL/min/1.73 m^2
    - Total bilirubin 0 – 21 µmol/L (0 -1.24 mg/dL)
  - Sirolimus is to be administered daily by mouth.
  - The drug may be given without regard to food, but should be consistently administered the same way (with or without food).
  - The oral solution may be diluted in 2 ounces of water or orange juice immediately prior to administration. The glass should be rinsed with an additional 4 ounces of the same fluid and administered to the patient.
  - Dose adjustments will not be made based on trough levels.

DRUG MODIFICATIONS FOR TOXICITY

If the patient suffers any Grade 3 or 4 toxicity that the treating physician feels may delay surgery, the patient will come off study.

Dose Modifications for Sirolimus

Toxicity	Criteria/Grade	Action
Myelosuppression	ANC < 0.75 x 10^9/L Plts < 75 x 10^9/L	Withhold sirolimus, and repeat within 3-4 days until neutropenia or thrombocytopenia are resolved. If no previous delay, resume at full dose. If previous delay, reduce dose by 25%.
Mucositis, Severe Abdominal Pain, Diarrhea	Grade 4 mucositis or repeated Grade 3 mucositis	Delay until resolved and decrease subsequent sirolimus dose by 25%.
Renal Toxicity	Serum creatinine > 2 x baseline or GFR < 70 mL/min/1.73 m2	Delay for one week and decrease subsequent sirolimus dose by 25%. If renal function does not improve, discontinue sirolimus.
Hepatic Toxicity	Raised Total Bilirubin	Reduce sirolimus as follows: **Bilirubin Concentration — % Dose** 0 – 21 µmol/L (0 -1.24 mg/dL) — 100% 22 – 35 µmol/L (1.25-2.09 mg/dL) — 75% 36 – 52 µmol/L (2.1 -3.05 mg/dL) — 50% 53 – 86 µmol/L (3.06-5.0 mg/dL) — 25% > 87 µmol/L (> 5.0 mg/dL) — 0%

Other Toxicity:
Any other persistent grade 3 or 4 toxicity should be discussed with the Study co-PIs and decided on a case by case basis. Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be used for toxicity assessment. Please see Adverse Event and Data Reporting for guidelines for submitting AdEERs forms to the NCI for other grade 4 toxicities.

AGENT INFORMATION

Sirolimus (Rapamune®, Rapamycin)

Supplier:

Commercially available as Rapamune® oral solution 1mg/mL and 0.5mg, 1mg, and 2mg tablets.

Pharmacology:

Sirolimus inhibits T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin (IL)-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Sirolimus also inhibits antibody production. Sirolimus binds to the immunophilin, FK binding protein-12 (FKBP-12). Sirolimus suppresses cytokine driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.

Formulation:

The 1 mg/mL oral solution must be refrigerated and protected from light. 0.5mg, 1mg, and 2mg tablets.

Stability:

Once the bottle is opened, the solution is stable for 30 days. The individual oral solution pouches are stable at room temperature for 24 hours. The bottles of oral solution are stable at room temperature for 15 days.

Guidelines for Administration:

Sirolimus is to be taken by mouth. Doses should be given every 24 hours +/- 2 hours. The drug may be given without regard to food, but should be consistently administered the same way (with or without food). The oral solution may be diluted in 2 ounces of water or orange juice immediately prior to administration. The glass used should be rinsed with an additional 4 ounces of the same fluid and administered to the patient.

Potential toxicities:

	Common (21-100% Frequency)	**Occasional (5-20% Frequency)**	**Rare (<5% Frequency)**
Immediate: Within 1-2 days of recovery drug	Nausea, Vomiting	Rash	Angioedema
Prompt: Within 2-3 weeks, prior to next course.	Diarrhea Hypertension Increased Creatinine	Infections (e.g. nasopharyngitis); Edema; Weight Gain, Arthralgia; Tremor; Acne; Myelosuppression; Abdominal Pain; Fatigue; Myalgia; Chest Pain; Dizziness; Hypokalemia; Hypophosphatemia; Hyperglycemia (diabetes mellitus)	Hepatotoxicity
Delayed: Anytime after above.	Hypercholesteremia, Hypertriglyceridemia	Stomatitis, Wound Complications	Hirsutism, Secondary Lymphoma, Pulmonary Hemorrhage Interstitial Lung Disease
Late: Anytime after completion of treatment			

TABLE OF CLINICALLY RELEVANT DRUG INTERACTIONS FOR CYP3A4, 5, 0r 7

SUBSTRATES	INHIBITORS	INDUCERS
Macrolide antibiotics: clarithromycin erythromucin NOT azithromycin Telithromycin **Anti-arrhuythmics:** alprazolam diazepam midazolam triazolam **Immune Modulators:** cyclosporine *tacrolimus (FK506)* **HIV Protease Inhibitors:** indinavir ritonavir saquinavir **Antihistamines:** astemizole chlorapheniramine **Calcium Channel Blockers:** amlodipine, diltiazem, felodipine, nifedipine, nisoldipine, nitrendipine, verapamil **HMG CoA Reductase Inhibitors:** Atorvastatin, cerivastatin, lovastatin, NOT pravastatin, simvastatin **Other:** aripiprazole buspirone cisapride imatinib haloperidol (in part) methadone pimozide quinine NOT rosuvastatin	**HIV Antivirals:** delaviridine indinavir nelfinavir ritonavir **Other:** amiodarone aprepitant NOT azithromycin chloramphenicol cimetidine clarithromycin diethyl-dithiocarbamate diltiazem erythromycin fluconazole fluvoxamine gestodene grapefruit juice itraconazole ketoconazole mifepristone nefazodone norfloxacin norfluoxetine mibefradil star fruit verapamil voriconazole	**HIV Antivirals:** efavirenz nevirapine **Other:** barbiturates carbamazepine glucocorticoids modafinil phenobarbital phenytoin rifampin St. John's wort troglitazone oxcarbazepine pioglitazone rifabutin

SUBSTRATES	INHIBITORS	INDUCERS
sirolimus sildenafil tamoxifen trazodone vincristine		

Adapted from a table prepared by Division of Pharmacology School of Medicine at Indiana University. New drug interactions may be identified after this table was printed; please check periodically for updates at http://medicine.iupui.edu/flockhart/

TABLE OF P-GLYOPROTEIN SUBSTRATES, INDUCERS, AND INHIBITORS

SUBSTRATES	INHIBITORS	INDUCERS
amiodarone amprenavir, (also indinavir, fosamprenavir, ritonavir, saquinavir, nelfinavir) atorvastatin, lovastatin celiprolol cetrizine cimetidine ciprofloxacin colchicines cyclosporine daunorubicin (also doxorubicin, idarubicin) desloratidine, loratadine dexamethasone digitoxin, figoxin diltiazem docetaxel erythromycin estradiol etoposide, teniposide fexofenadine hydrocortisone imatinib irinotecan ivermectin lidocaine loperamide methotrexate mitomycin	amiodarone amitriptylinie, (also desipramine, imipramine, trimipramine) atorvastatin, (also lovastatin, simvastatin) azelastine carvedilol, propanolol chlorpromazine prochlorperazine cimetidine, ranitidine clarithromycin, erythromycin cyclosporine dexrazoxane diltiazem, felodipine (also nicardipine, nifedipine, nitredipine, verapamil) dipyridamole disulfiram doxepin esomeprazole (also lansoprazole, omperazole) fluphenazine grapefruit juice haloperidol hydrocortisone hydrozyzine imatinib itraconazole, ketoconazole ivermectin	Aspirin dexamethasone doxorubicin nefazodone prazosin rifampin St. John's wort Trazodone vinblastine

SUBSTRATES	INHIBITORS	INDUCERS
nadolol nicardipine ondansetron paclitaxel pravastatin quinidine ranitidine rifampin sirolimus tacrolimus verapamil vinblastine, vincristine	lidocaine marprotilinie mefloquine midazolam mifepristone mitomycin nefazodone nelfinavir (also ritonavir, saquinavir) ofloxacin probenicid progesterone propafenone quinidine, quinine reserpine rifampin tacrolimus tamoxifen testosterone vinblastine	

Note: Predictions of drug interactions due to an effect on PGP transport is limited by the fact that drugs may be metabolized by multiple pathways, offering an alternative elimination route; the sum of the multiple drug effects on PGP is unknown; and PGP activity is influenced by non-drug entities such as: inflammation, irradiation, etc.
Adapted from Lexi-Comp's Drug Interaction Handbook 2nd edition 2004

CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Criteria For Removal From Protocol Therapy

- Progressive disease
- Dose-limiting toxicity
- Patient withdrawal from protocol
- A delay in surgery > 7 days from the completion of sirolimus
- Patient missed >25% of sirolimus doses

Patients who are off protocol therapy are to be followed until they meet the criteria for off study (see below). Follow-up data will be required.

Off Study Criteria

- Death
- Lost to follow-up
- Patient withdrawal of consent for study follow-up

PATHOLOGY GUIDELINES

Pre-study pathology:
Pre-Study tumor or biopsy sample will be analyzed for confirmation of diagnosis and for immunohistochemical analysis for mTOR pathway activation.

To accomplish this, representative H and E-stained sections and six (6) unstained, 5 micron sections on positive-charged slides, cut from the same block will be submitted. If slides cannot be sent, blocks would be acceptable.

Pathology of the specimen after neo-adjuvant chemotherapy:
Immunohistochemical studies for mTOR pathway activation will be performed on the resected tumor samples. Immunostaining results will be compared to a cohort of previously untreated, archived specimens, and also paired pre-treatment tumor or biopsy specimens if available. (See more below in Statistical Analysis.)

To accomplish this, representative H and E-stained sections and ten (10) unstained, 5 micron sections on positive-charged slides, cut from the same block will be submitted. If slides cannot be sent, blocks would be acceptable.

Pathology specimens should be sent within 3 weeks of enrollment to:

Sarah Dry, MD
UCLA Department of Pathology
Bone, Soft Tissue and GI Pathology
Director, Translational Pathology Core Laboratory, 13-145 CHS
10833 Le Conte Ave, Los Angeles, CA 90095
Phone: 310.794.9311
Fax: 310.267.2104

Histological Assessment

H and E staining will be used to verify that the arrayed tissue/slides contain desmoid-type fibromatosis. To assess mTOR pathway activation, immunohistochemical staining will be conducted for the following proteins and phosphoproteins: p-p70S6K, p-4E-BP1, both of which represent targets of mTOR; and p-AKT as this pathway could be activated as a drug resistance mechanism (see Figure 1). Staining will be performed on the (a) post-treatment specimens, (b) paired, pre-treatment, archived specimens (if available), and (c) archived non-chemotherapy-treated specimens from the UCLA tumor bank (approximately 50 are available). All samples will be anonymized using an alpha-numeric code, with the key maintained in a HIPAA-compliant, secure database or locked file on the UCLA Server.

Routine histology protocols (including positive and negative controls) will be used. Quantitative analysis will be accomplished utilizing an Immuno-Reactive Score (IRS) as previously described (Noske et al., 2008; Chen et al., 2009; Lin et al., 2006). Specifically, the percentage of positive cells will be scored as: 0 (0%); 1 (<10%); 2 (11-50%); 3 (51-80%); 4(>80%). The staining intensity will be scored as: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). To derive the IRS, the percentage of positive cells and staining intensity will be multiplied together, resulting in a value from 0 to 12. IRS values 0 to 3 will be considered negative and values of 4 to 12 will be considered positive. Of note, samples will be scored by an experienced pathologist, blinded to whether the samples represent pre- or post-treatment or archived non-chemotherapy treated specimens.

Pain Assessment

Validated pain assessment measures, using the numeric and Wong-Baker FACES scales, will be collected at specific study time points. Patients ≥3 and < 10 years of age will use the Wong-Baker FACES scale and patients who are ≥ 10 years of age will use the numeric scale. A positive response will be associated with an improvement of ≥ 50% from baseline after 4 weeks. A negative response will be associated with any worsening from baseline after 4 weeks. A stable response will be any other result.

Radiographic Assessment

Anatomical imaging will be performed before therapy, prior to surgery (optional but strongly recommended), and at designated post-surgery surveillance intervals. Although tumor size will be recorded at all imaging time points and compared, the main purpose of imaging will be to detect recurrence following treatment. The slow growing nature of desmoid tumor precludes the use of previously established standardized radiographic measurements of response, such as the RECIST, for patients participating in this pilot study.

RESPONSE CRITERIA

Disease Response

Disease response is defined as follows:

Complete Response (CR)
No clinical or radiographic evidence of disease

Partial Response (PR)
Reduction in the greatest product of 2 perpendicular diameters by >50%, no new lesions.

Minor Response (MR)
Reduction in the greatest product of 2 perpendicular diameters by >25%, but ≤50%, no new lesions.

Stable Disease (SD)
Change in the greatest product of 2 perpendicular diameters by ≤25%, no new lesions.

Progressive Disease (PD)
Increase in the greatest product of 2 perpendicular diameters by >25% or new biopsy-proven lesions.

RISKS AND BENEFITS

Specific drug risks have been included in the Agent Information section of the protocol.

Blood drawing may cause pain, bruising, bleeding, or infection at the site of the needle stick. Care will be taken to avoid these complications. There should be no added risks to subjects from the blood drawing or surgical procedures as a result of participating in this study.

Another risk to subjects is the release of information from health records. Sometimes, health records have been used against subjects and their families. For example, insurance companies

may deny a patient insurance or employers may not hire someone with a certain illness (such as AIDS or cancer). We will protect subjects' health records so that their name, address, and phone number will be kept private. The chance that this information will be given to someone else is very small.

The protocol therapy may or may not provide benefit to participating subjects. We hope that information learned from this clinical trial will benefit future desmoid tumor patients.

INFORMED CONSENT PROCEDURE

The subject or subject's guardians are first presented with the opportunity to join the trial in the Hematology/Oncology clinic at the respective institution when it is determined that the subject meets all protocol eligibility criteria. The aim of the study will be explained to the subject or the subject's guardians in non-technical language by the principal investigator or by any of the members of the pediatric oncology team listed in supplemental form A. Once the subjects consent to the study they are told that they may contact Dr. Weiss, Dr. Federman, Dr. Sharma or other collaborating physicians and nurses involved with the study to ask any additional questions or concerns that they may have. Also, if the subject consents to the study, throughout the course of the study, the treating physician and nurses will continue to explain the nature of the study in non-technical language. Assent will be sought from pediatric patients who are of an appropriate age and cognitive status.

SUBJECT CONFIDENTIALITY

The Private Health Information (PHI) for pre-screening case selection includes: subjects' name, medical record number and surgical pathology report. There will be potential risk resulting from breach of confidentiality. After clinical information is abstracted on each case, all clinical information and material for analysis will be de-identified and given a unique case number. Data will be abstracted onto a secure database. The link between the study code and the subject identifiers will be retained separately from the study documents in a secured password protected database only accessible to the research staff.

All patient records used for this study will be kept confidential. Patients will not be identified by name in any publications describing the results of this study. Access to the patient records will contain identifiers that will be made available to the Principle Investigator and Clinical Research Associate.

A database will be maintained by the principal investigator at the primary clinical trial site (MMC/MCCP). The computer as well as the database will be password protected.

ADVERSE EVENT AND DATA REPORTING

An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease deemed to be possibly related to or occurring during the use of an investigational product.

Serious adverse events (SAE) are adverse events occurring at any dose which meet one or more of the following serious criteria:

- Results in death (i.e. the AE caused or lead to death)
- Is life-threatening (i.e. the AE placed the subject at immediate risk of death; it does not apply to an AE which hypothetically might have caused the death if it were more severe)
- Requires or prolongs inpatient hospitalization (i.e. the AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion)
- Is disabling (i.e. the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a subject exposed to the study drug prior to conception or during pregnancy)
- It does not meet any of the above serious criteria but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

When assessing causality, the investigator should determine the association and consider if there is a clinically plausible time sequence between onset of the AE and the associate drug administration; and/or there is a biologically plausible mechanism for the drug causing or contributing to the AE; and the AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

Expected adverse events are those adverse events that are listed or characterized in the Package Insert. Unexpected adverse events are those not listed in the Package Insert or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the Package Insert.

Unexpected adverse events grade 3, 4 and 5 as well as any medical event equivalent to CTC Grade 3, 4 or 5, which precipitated hospitalization (or prolongation of existing hospitalization) will be reported regardless of designation as expected or unexpected, along with attribution, to Dr. Aaron Weiss's pager at 207-741-6679 **within 24 hours**. These reportable adverse events will also be documented on a Medwatch 3500A form supplied by the coordinating center. The Medwatch 3500A form will be submitted to Dr. Aaron Weiss within the time frame specified under AE Reporting Guidelines (on page 24) via fax to 207-396-7577 – use a FAX cover page.

Expected adverse events grade 4 and 5 will be reported on a study supplied Medwatch 3500A form **within 5 working days or 10 calendar days, whichever is sooner**. (use study supplied Medwatch 3500A form).

Dr. Aaron Weiss, who will discuss the adverse event with the local principal investigator (PI), will ensure that the Medwatch form has been obtained and the adverse event reported to the independent reviewer, Christian Thomas MD, who has been designated as independent data safety medical monitor. Christian Thomas, MD, Director of Research at Maine Center for Cancer Medicine will review incoming adverse event information and any data in real time. The local principal investigator or treating physician along with Dr. Aaron Weiss, will determine if an AE is treatment related. Dr. Thomas will review and evaluate all incoming events for causality and may discuss the events with all the PIs. Dr. Thomas will review attribution and causality and may disagree with the previous determination. Documentation of his review and any of the previous reviews will be forwarded to the bi-monthly Pediatric Solid Tumor Board. Dr. Thomas has the authority to determine if an adverse event requires the study to be discontinued or be put on hold. If this occurs, all participating sites will be notified immediately as well as the MMC IRB. All sites are expected to inform their local IRB.

In addition, patients enrolled to this study will be regularly discussed as a part of the bi-monthly Pediatric Solid Tumor Board. The discussion will include tumor response, toxicity and reported adverse events. A copy of all adverse event documentation and a summary of the discussion between Dr. Aaron Weiss and independent reviewer will be given to the Tumor board. The bi monthly Pediatric Solid Tumor Board Conference has the authority to determine if an adverse event requires the study to be discontinued or put on hold. A Data and Safety Monitoring worksheet will be completed at this conference.

The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 4.0 (http://ctep.cancer.gov/reporting/ctc.html) will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTC version 4.0.

A table showing the **expected** adverse events specific for each of the agents is found below.

Expedited Adverse Event Reporting

*Expedited Reporting Guidelines – (including **hospitalization** defined in bullet 1 below)*

Adverse Event Reporting Guidelines

Unexpected Event	**Expected Event**	
Grade 3, 4 and 5	Grade 3	Grade 4 and 5
Report by phone to MMC/MCCP Coordinating Center Dr. Aaron Weiss at 207-741-6679 within 24 hrs. Written expedited Medwatch Form 3500A report to be faxed to 207-396-7677 within 48 hours. This includes all deaths within 30 days of the last dose of sirolimus treatment regardless of attribution. Any late death attributed to sirolimus (possible, probable, or definite) should be reported within 48 hours of site's knowledge by phone and Medwatch form per above.	Adverse Event Expedited Reporting NOT required.	Written Medwatch 3500A report, within 5 working days or 10 calendar days, whichever is sooner. This includes all deaths within 30 days of the last dose of sirolimus treatment regardless of attribution. Any late death attributed to sirolimus (possible, probable, or definite) should be reported within 48 hours of site's knowledge by phone and Medwatch form per above.

For **Hospitalization** only – Any medical event equivalent to CTC Grade 3, 4, or 5, which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.

Written reported procedures:
• A completed MedWatch form will be utilized for collecting adverse event information. Complete in the timeframe specified in guidelines above and fax to 207-396-7577 to the attention of Dr. Aaron Weiss. In addition each site must report adverse events per their local IRB policy.

All delinquent reporting to the MMC/MCCP coordinating center must include documentation of reason for delinquency and may require implementation of an action plan.

Records, Reporting, and Study Monitoring –
Data Collection Forms:
Forms should be completed at specified time points and sent to:

Kathleen M. Glick, CCRP
Coordinator of Clinical Research
Maine Children's Cancer Program
100 Campus Drive, Unit 107
Scarborough, ME 04074
Phone: 207.396.7565
Fax: 207.396.7577
Email- glickk@mmc.org

Monitoring of Study Data:

As the coordinating center MMC/MCCP will monitor and review all data for accuracy and completeness, and will collect data quarterly. Queries will be generated to clarify unclear or missing data. Records of this review will be maintained at MMC/MCCP. Eligibility, deviations, adverse events and essential data elements will routinely be verified. As the coordinating center MMC/MCCP will obtain a copy of each local sites IRB approval and IRB approved informed consent documents and all subsequent IRB reportable changes. On an annual basis a random review of 20% of the data not previously reviewed will be performed.

Unidentified patient SAE data will be shared w/ Pfizer.

STATISTICAL ANALYSIS

Primary Objective

The primary scientific endpoint for patients enrolled on this pilot study is determine whether mTOR pathway activation decreases in patients with surgically-resectable desmoid tumor that is removed following pre-operative treatment with sirolimus. We anticipate finding that 4 weeks of sirolimus will result in decreased mTOR pathway activation, assessed using immunohistochemical (IHC) studies outlined above. IHC results after 4 weeks of sirolimus, scored on a scale of 0-12, from patients enrolled on the study will be compared with archived tumor samples from untreated patients using a two-sample t-test. The sample size of n=15 trial patients and n=50 historical controls will provide 80% power to detect a true difference in means of 2.6 points on the 12-point scale. While the t-test is fairly robust, if the data are highly non-normally distributed a nonparametric, rank-sum test will be applied instead. In addition, responses will be dichotomized as described above (negative <3, positive >4) and the

proportion positive compared between the two groups using Fisher's exact test. Finally, pre- and post-treatment values within patients enrolled on the trial will be compared using paired t or Wilcoxon, signed-rank tests.

Secondary Objectives

These scientific endpoints will be mostly exploratory, given the small numbers of patients enrolling in this pilot study. Nonetheless, we anticipate finding that 4 weeks of sirolimus will result in decreased tumor-associated pain. Pain assessments will be made using validated age-based pain assessment scales and compared pre- and post-treatment using a paired t test. Descriptively, the number of patients showing >50% improvement in their pain score, worsening pain, or between 0 and 50% improvement will be tabulated. Having decreased pain in approximately 60% of patients without substantial toxicity will be viewed as a success.

Patients will also be followed for recurrence post-surgery. As a benchmark, in a similar cohort of patients treated on the Pediatric Oncology Group 9650 study with vinblastine and methotrexate, the progression-free survival rate at 1 year was 58%. Vinblastine and methotrexate, which most would agree represents the current standard of care for desmoid tumor that is not amenable to surgery or radiation, is associated with significant toxicity: 18 of 27 (66%) patients on that trial experienced NCI grade 3 or 4 toxicity. Having histochemical evidence for decreased mTOR pathway activation with less toxicity will be viewed as a success, and lead the investigators to explore ways to test sirolimus in a randomized, phase II study in which the drug is used for patients with unresected disease and for patients in which the drug is continued following surgical resection of disease.

An independent statistician has been recruited from Center for Outcomes Research and Evaluation (CORE) at Maine Medical Center Research Institute. Lee Lucas, PhD, assisted in the original presentation of this project. There will be no interim analysis performed; analysis will be performed at study completion. If Lee Lucas is not available, Christine Duarte, PhD. Senior Biostatistician at CORE, will be the lead statistician for this project. Dr. Duarte will be assuming the analysis or consulting on this project as this is her area of expertise and her background is in translational research.

Early Stopping Rules

We anticipate surgical complications – such as excessive bleeding or wound dehiscence – to be rare, occurring in no more than 1 of the 15 patients enrolled. If these or other significant and unexpected surgical complications are found in 3 patients, the study will be terminated.

We feel that Grade 3 or 4 toxicity that will delay surgery will be rare, occurring in no more than 1 of the 15 patients enrolled. If this occurs in 3 patients, the study will be terminated.

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APPENDIX 1

Wong-Baker FACES Scale
(to be used with patients >3 and <10 years of age)

Numeric Pain Scale
(To be used with patients ≥10 years of age)