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Sirolimus for Non-Progressive NF1-Associated Plexiform Neurofibromas: An NF Clinical Trials Consortium Phase II Study

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Background. Patients with Neurofibromatosis Type 1 (NF1) have an increased risk of developing tumors of the central and peripheral nervous system, including plexiform neurofibromas (PN), which are benign nerve sheath tumors that are among the most debilitating complications of NF1. There are no standard treatment options for PN other than surgery, which is often difficult due to the extensive growth and invasion of surrounding tissues. Mammalian Target of Rapamcyin (mTOR) acts as a master switch of cellular catabolism and anabolism and controls protein translation, angiogenesis, cell motility, and proliferation. The NF1 tumor suppressor, neurofibromin, regulates the mTOR pathway activity. Sirolimus is a macrolide antibiotic that inhibits mTOR activity. ***Procedure.*** We conducted a 2-stratum phase II clinical trial. In stratum 2, we sought to determine whether the mTOR inhibitor sirolimus in subjects with NF1 results in objective radiographic responses in inoperable PNs in the absence of documented radiographic progression at trial entry. ***Results.*** No subjects had better than stable disease by the end of six courses. However, the children's self-report responses on health-related quality of life questionnaires indicated a significant improvement in the mean scores of the Emotional and School domains from baseline to 6 months of sirolimus. ***Conclusions.*** This study efficiently documented that sirolimus does not cause shrinkage of non-progressive PNs, and thus should not be considered as a treatment option for these tumors. This study also supports the inclusion of patient-reported outcome measures in clinical trials to assess areas of benefit that are not addressed by the medical outcomes. Pediatr Blood Cancer 2014;61:982–986.

Key words: clinical trials; mTOR; neurofibromatosis; new agents; solid; tumors

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder with an incidence of 1:3,000 worldwide (>80,000 persons affected in the United States alone). NF1 is caused by a germline mutation in the *NF1* tumor suppressor gene located on chromosome 17q11.2. Patients with NF1 have an increased risk of developing tumors of the central and peripheral nervous system including plexiform neurofibromas (PN) [1,2].

PNs are benign peripheral nerve sheath tumors that grow along the length of nerves, and involve multiple fascicles and branches of a nerve. These tumors are usually diagnosed early in life, may be multiple in number, and can grow throughout life, though early childhood is considered to be the period of greatest risk for disease progression [3,4]. Between 20–44% of individuals with NF1 develop PNs [5–7]. While considered "benign," these tumors may cause significant disfigurement, as well as compression of vital structures. For example, PNs may infiltrate the orbit and are associated with orbital dysplasia, displace the globe and compromise vision; paraspinal tumors can compress the spinal cord and cause paralysis; tumors in the mediastinum may compress the trachea or great vessels; and tumors of the extremities can cause progressive neurologic deficit and significant pain [3,8], all of which can impair daily functioning and quality of life (QOL). The management of PNs is difficult, and currently, there is no acceptable effective drug therapy. Thus, the only curative management is surgical resection. However, resection is challenging due to the infiltrating nature of the tumors and their significant vascularity. In addition, there is a high risk of tumor progression after the first surgery [3,9].

There is a great need for the development of targeted therapies for inoperable PN in NF1. The NF1 tumor suppressor, neurofibromin, regulates mTOR pathway activity, which is activated in *NF1*-deficent cells and tumors from NF1 patients [10,11]. mTOR is a serine/threonine kinase that acts as a central integrator of many cellular functions, including cell size/growth, proliferation via modulation of cell cycle transit, survival, translation, metabolism, autophagy, angiogenesis, and aging; it does so by responding to the availability of nutrients, glucose, and amino acids, as well as growth factors and stress signals from the microenvironment [12]. mTOR regulates these processes in part by controlling the protein translation machinery [13], which increases the synthesis of nutrient and amino acids transporters as well as key pro-growth and survival molecules, such as HIF-1α, cyclin D1, and myc [14]. Sirolimus, an allosteric inhibitor of mTORC1, is an effective immunosuppressant, currently FDA-approved as an anti-rejection medication for solid organ and bone marrow transplant [15]. Dosing in young children is both well established and safe over long periods. Recent studies have demonstrated that the *NF1* tumor suppressor regulates mTOR pathway activation and that abrogating

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the activity of this pathway by inhibiting mTOR function reduces tumor proliferation in *Nf1* genetically engineered mouse models and human NF1-associated tumor explants [10,16,17].

To explore the role of mTOR inhibition in the treatment of NF1-associated plexiform neurofibroma, we conducted a 2-strata phase II clinical trial. We sought to determine whether the mTOR inhibitor sirolimus in subjects with NF1 either (a) increases time to progression (TTP) in progressive PN (stratum 1) or (b) results in objective radiographic responses in inoperable PNs in the absence of documented radiographic progression at trial entry (stratum 2). Herein we report the results of subjects enrolled in Stratum 2. Volumetric magnetic resonance imaging (MRI) analysis conducted at regular intervals was used to determine response or progression relative to baseline imaging. We also evaluated the effects of treatment with sirolimus on pain and QOL using patient-reported outcome (PRO) measures.

METHODS

Study Design and Population

Subjects were enrolled at one of nine Department of Defense funded NF Clinical Consortium sites. Inclusion criteria included age $\geq$3 years with a diagnosis of NF1 and an unresectable PN with the potential to cause significant morbidity, such as (but not limited to) head and neck lesions that could compromise the airway or great vessels, brachial or lumbar plexus lesions that could cause nerve compression and loss of function, lesions that could result in major deformity, lesions of the extremity that cause limb hypertrophy or loss of function, and painful lesions. Subjects with paraspinal PNs were eligible for this trial. Histologic confirmation of the tumor was not necessary in the presence of consistent clinical and imaging findings. PNs had to be amenable to volumetric MRI analysis. Subjects may have been previously treated, but must have recovered from the acute toxic effects of all therapy. Other eligibility criteria included (a) adequate performance status (Lansky score of 50 or more); (b) normal blood count and renal, liver, and cardiac function; and (c) signed informed consent from the patient, parent, or guardian, according to institutional review board guidelines. Subjects with progressive PN (defined as a new PN or based on volumetric [$\geq$20% in volume], area [$\geq$13% in product of two longest perpendicular diameters], or linear [$\geq$6% in longest diameter] increase in PN size on two MRI scans done within 1 year) were enrolled on Stratum 1. All other eligible subjects without evidence of progression, which included three subjects followed for less than 1 year, were enrolled on Stratum 2.

Subjects were excluded if they (a) had received prior therapy with an mTOR inhibitor; (b) had evidence of a tumor or cancer requiring treatment with chemotherapy or radiation; (c) were post-pubertal males/females who would not agree to use effective contraception, or were pregnant/breast-feeding females; (d) had a known history of HIV or immunodeficiency; (e) had impairment of gastrointestinal function or gastrointestinal disease that significantly alters the absorption of sirolimus; (f) had dental braces or prosthesis that interfere with volumetric analysis; or (g) had concurrent severe and/or uncontrolled medical disease. In subjects who had received therapy prior to study enrollment, 2 weeks-6 months must have elapsed, depending on the specific prior therapy. Corticosteroid use was not allowed while on study, nor was the use of strong CYP3A4 inducers/inhibitors.

Therapy

The starting dose of sirolimus (1 ml = 1 mg) was 0.8 mg/m^2 by mouth twice daily. The starting dose was based on extrapolation of the recommended sirolimus dose in older children and adult transplant recipients [18]. Subsequent dosing was pharmacokinetically guided to achieve a trough blood concentration of 10–15 ng/ml; this target range was based on an effective sirolimus target reported in patients with Tuberous Sclerosis Complex (TSC)-associated subependymal giant cell astrocytoma [19]. The first sirolimus concentration measurement was performed at steady-state following 7–10 days of treatment. Subsequent trough concentrations were obtained every 2 weeks until stable, which was defined as two consecutive trough concentrations between 10 and 15 ng/ml. Once the dose was stable, trough concentrations were measured every course (course = 28 days).

Sirolimus whole blood concentrations were centrally determined by a validated tandem mass spectrometry assay, as previously described [20]. Lab results were reported using a web-based protocol management tool, and included real-time dosing recommendations generated with a Bayesian estimator (MW/Pharm version 3.6, Mediware, Groningen, Netherlands) [20]. The system automatically generated email notifications to the clinical teams, which included the recording of acceptance of dosing adjustments.

The pediatric population parameter estimates for PK-guided dosing were derived from published pharmacokinetic data in stable renal transplant patients treated with sirolimus [20]. The patient demographics, dosing and concentration-time data sets entered into the MW/Pharm program were used for the Bayesian estimation.

On Study Evaluations

Subjects had non-contrast axial and coronal STIR MRI obtained at enrollment and after every three courses until course nine, then after every six courses of therapy. Volumetric analysis was performed centrally at the NCI, as previously reported [4,21]. Subjects who did not experience a partial response to sirolimus after six courses were removed from protocol therapy. Toxicity was monitored with a physical examination and laboratory studies (including blood count and comprehensive metabolic panel) monthly for the first three courses, then every three courses. Those subjects who consented (and their parents if they were $<$18 years old) also completed assessments of health-related QOL and pain intensity at the same interval of MRI testing using the following methods.

PRO Measures

General health-related quality of life (HRQOL). The *PedsQL 4.0 Generic Core Scales* is a reliable and valid 23-item questionnaire assessing four domains (physical, emotional, social, and school functioning) of HRQOL in children 3 years old through adults [22,23]. Subjects, ages 5–25 years, completed the self-report forms and the parents of children, ages 3–18 years, were administered the parent proxy form. Items rated on a five-point Likert scale (0–4) are transformed to a 0–100 scale, and higher domain and total mean raw scores indicate better QOL. The *Functional Assessment of Cancer Therapy-General* (FACT-G) is a reliable and valid 27-item measure of general HRQOL, assessing 4 generic core subscales (physical, emotional, social, and functional

well-being) in adults 25 years and older with cancer and other chronic illnesses [24,25]. Items are rated on a five-point Likert scale (0–4), and higher subscale and total *T*-scores (Mean = 50; SD = 10) indicate better QOL.

Pain intensity. Parents and children from 5 to 18 years of age independently rated the intensity of the child's worst pain during the past week on the visual analogue scale (VAS) from the *PedsQL Pediatric Pain Questionnaire* [26]. This VAS consists of a happy face and "no pain" written on the left end of the line and a sad face and "severe pain" written on the right end. The child or parent is instructed to put a mark on the line that indicates how much pain the child is feeling. Adults, ages 18 years and older, rated the intensity of their worst pain during the past week on the VAS from the *Short-Form McGill Pain Questionnaire* [27]. Similar to the PedsQL VAS, the McGill VAS consists of a line with "no pain" written at the left end of the line and "worst possible pain" at the right end. For both of these scales, the score is the distance of the mark from the left end of the line, measured in millimeters (0–100 mm). Higher scores indicate greater pain intensity. VAS ratings are reliable and valid measures of pain intensity in both children [28] and adults [29].

Response and Toxicity

For response determination, volumetric measurements on follow-up scans were compared to the pretreatment PN volume. Complete Response (CR) was defined as complete resolution of all measurable PNs. Partial Response (PR) was defined as a $\geq$20% reduction in the sum of the volume of all index PN lesions, while Stable Disease (SD) was defined as $<$20% increase and $<$20% decrease in the sum of the volume of all index PN lesions. Progressive Disease (PD) was defined as $\geq$20% increase in the volume of at least one of the index PN lesions compared to the pretreatment volume measured. The appearance of new discrete subcutaneous neurofibromas did not qualify for disease progression, nor did the occurrence of new or increased symptoms without MRI evidence for progression. Subjects who completed at least one course of therapy were considered evaluable for response.

Toxicity was monitored using CTCAE version 3. Subjects were considered evaluable for toxicity if they received $\geq$ one dose of sirolimus and had a "severe" sirolimus-associated toxicity or, in the absence of a "severe" toxicity, completed one full course of therapy. Severe sirolimus toxicity was defined as any of the following toxicities identified during the first two courses: Grade $\geq$3 opportunistic infection/pneumocysitis jiroveci pneumonia, Grade $\geq$2 pneumonitis, Grade 4 rash, Grade $\geq$3 hypertension, Grade $\geq$3 allergic reaction, worsening renal function, or the development of lymphoma or other cancers.

Statistical Analysis

An optimal Simon two stage design with a null response rate of 0.05 and an alternative of 0.20 was used. The type 1 and type 2 error rates were both set at 0.10. This called for a first stage sample size of 12 with an additional 25 to be accrued if any of these 12 achieved at least partial response. In that case, sirolimus would be deemed effective if four or more of the 37 achieved at least a partial response.

The general QOL and VAS pain data were summarized with descriptive statistics (means, SD, and ranges). Paired *t*-tests were used to assess change, from the baseline to final PRO evaluation at course 6, in the mean scores of the child self-report and parent proxy PedsQL, adult self-report FACT-G, and the overall ratings of pain on the Visual Analog Scales. Due to the small sample size, the number of subjects who demonstrated stable functioning ($<$5 points) and clinically meaningful improvement or decline ($\geq$5 points) in their individual HRQOL (PedsQL or FACT-G) scores [30–32] was presented. On the VAS, the number of subjects who showed stable or clinically meaningful change of at least 30 mm in their ratings of pain intensity also was noted [33,34].

RESULTS

Subject Characteristics

Of the 13 subjects enrolled on Stratum 2 (mean age 16 years, range 3–35 years; Table I), one subject withdrew consent and never took study drug; therefore, a total of 12 subjects were evaluable for response or toxicity. While lack of progression was documented in all subjects at enrollment, three subjects had an observation period with PN measurements for less than 12 months. Eleven subjects signed consent to participate in the PRO studies evaluating QOL and pain. Of these, six children and their parents completed the PedsQL QOL scale and the pain VAS (one child was too young for the self-report form and had disease progression prior to course 3, and one subject had incomplete follow-up data), and three adults completed the FACT-G QOL scale and the McGill pain VAS at baseline and course 6. Thus, nine subjects (six children mean age = 11.0 years; range 5–17 years and three adults mean age = 29.3 years; range 25–34 years) had complete PRO data.

Outcomes

No subjects had better than SD by the end of course 6 (Table II). One subject had documented PD. All subjects achieved target concentration by course 3 (mean 2.1 courses; range 1–3 courses). By design, since none of the first 12 subjects had a response by course 6, this stratum was closed without rejection of the null hypothesis.

TABLE I. Subject Demographics

Participant's characteristics	STRATUM 2 N (%)
Sample size	
Total	13
Age in year	
Mean	16
Min	3
Max	35
Median	16
Race	
White	10 (76.9)
Black, African American	2 (15.4)
Native Hawaiian, Other Pacific Islander	0 (0.0)
Asian	1 (7.7)
American Indian or Alaska Native	0 (0.0)
Ethnicity	
Hispanic or Latin	1 (7.7)
Non-Hispanic	12 (92.3)
Sex	
Male	5 (38.5)
Female	8 (61.5)

TABLE II. Plexiform Neurofibroma (PN) Characteristics

Subject	Location	PN volume at enrollment (ml)	Percent change in volume from baseline to post course 6
1	Head/neck/chest	2,476	+7.2
2	Pelvis/buttocks	802	+2.0
3	Neck/chest/arm	959	+6.7
4	Face	23	+3.5
5	Paraspinal	37	+0.5
6	Thigh/buttocks	1,246	−4.0
7	Paraspinal	173	+8.1
8	Neck/chest	892	−3.6
9	Paraspinal/pelvis/thigh	1,458	−0.6
10	Paraspinal/pelvis	392	−3.1
11	Chest	34	−7.4
12	Abdomen/pelvis	916	+21.5

General QOL. For the children (n = 6) who completed the PedsQL self-report form, there was no significant change in the mean Total Scores from baseline to course 6 (60.15–71.56; mean change = 11.41; $t = 1.74$, $P = 0.14$). Examining each child's individual data, three had stable Total Scores (<5 points change) and three had scores that improved ≥5 points. Analyses of each domain indicated no change in the mean Physical (68.75–79.17; mean change = 10.42; $t = 1.29$, $P = 0.2545$) or Social (58.33–59.17; mean change = 0.83; $t = 0.044$, $P = 0.9669$) Domain Scores from baseline to 6 months. However, the mean Emotional (55.83–74.17; mean change = 18.33; $t = 2.86$, $P = 0.0354$) and School (52.50–69.17; mean change = 16.67; $t = 4.663$, $P = 0.0055$) Domain Scores showed a significant increase at 6 months. Examination of individual scores in these two domains indicated that five children's scores improved in the Emotional Domain (one subject's score was stable), while all six children's scores improved in the School Domain.

For the parents who completed the PedsQL proxy form about their child's QOL at baseline and after course 6, there was no significant change in the mean Total Scores (63.10–61.23; mean change = −1.88; $t = -0.7076$, $P = 0.5108$) or any of the mean Domain Scores. Examining the individual scores, three parent proxy Total Scores were stable, two declined ≥5 points, and one improved ≥5 points.

For the three adults completing the FACT-G, there was no significant difference in mean Total Scores from baseline to course 6 (45.33–41.47; mean change = −3.87; $t = -1.73$, $P = 0.2264$) or any of the Domain Scores. Examining the individual FACT-G Total Scores, two adults showed improvement and one had a decline (all > 5 points).

Pain Intensity. At baseline, seven out of the nine subjects rated some level of pain on the VAS. There was no significant change from baseline to course 6 in the mean pain ratings of these nine subjects (43.72–36.93; mean change = −6.79; $P = 0.5699$) or of the six parents (51.52–35.39; mean change = −16.13; $t = -.79$, $P = 0.4655$) completing the VAS on their child. A review of the individual pain ratings found that from baseline to 6 months, three subjects had a clinically meaningful decrease and one had an increase of at least 30 mm while five remained stable. For the parents, two rated their child's pain as decreasing, one as increasing, and three as stable over the 6 months.

Toxicity

None of the subjects developed severe sirolimus toxicity, and none of the subjects required a target level reduction due to toxicity. One subject developed Grade 3 diarrhea in course 3 at least possibly related to sirolimus, which fully resolved. Other toxicities at least possibly related to sirolimus included triglyceride elevation in one subject, hypertension in one subject, headache in two subjects, cytopenia in two subjects, and moderate mucositis in four subjects (33%), all of which resolved while continuing sirolimus.

DISCUSSION

In 12 subjects, the mTOR inhibitor sirolimus, dosed twice daily to achieve a target blood level of 10–15 ng/ml, did not shrink non-progressive NF1-associated PNs after six courses of therapy. Sirolimus was tolerable in subjects with NF1 at doses similar to those administered in the transplant setting, and no subjects had target reductions due to toxicity. While the treatment duration was relatively short, target sirolimus concentrations were achieved in all subjects after three courses; therefore, inadequate sirolimus concentrations are not a potential reason for sirolimus inactivity. The lack of PN shrinkage in our trial is consistent with a recently described preclinical mouse neurofibroma model where administration of the mTOR inhibitor RAD001 did not result in tumor shrinkage [35]. In contrast, in a genetic NF1 MPNST mouse model mTOR inhibition resulted in substantial delay of tumor progression [36]. Whether sirolimus will prolong time to progression in those patients with progressive PNs at study entry is the subject of the other stratum on this study. That stratum has recently closed to accrual, and the results will be presented in a future publication.

This study used PRO measures to assess the clinical effects of sirolimus. In our small sample, no consistent group changes were demonstrated in pain intensity VAS ratings or overall QOL scores as reported by children, adults, or parents. However, the children's self-report measures, completed separately at baseline and after 6 months of sirolimus, indicated a significant improvement in the mean scores of the Emotional and School domains. Interestingly, a study evaluating the effects of tipifarnib, a farnesyltransferase inhibitor, in children with NF1 and PNs also found a significant improvement in the emotional domain scores as well as an upward trend in the cognitive domain scores of the Impact of Pediatric Illness Scale as rated by parents in the treatment arm compared to the placebo arm (Widemann, submitted). It is possible that sirolimus, and other drugs acting on the RAS pathway, may affect emotional and cognitive functioning. Evidence from both animal [37] and human studies [38] has shown that sirolimus may decrease depressive-like behavior and enhance learning and memory, possibly through the stimulation of major monoamine pathways in the brain [37]. Due to the small sample size, caution should be used in interpreting these preliminary PRO results, and additional studies will be required to further investigate this hypothesis. This study also supports the inclusion of PRO measures in clinical trials to assess areas of benefit that are not addressed by the medical outcomes [39].

This first NF Clinical Trials Consortium study directed at PNs efficiently documented that sirolimus does not cause shrinkage of

non-progressive PNs, and thus should not be considered as a treatment option for these tumors. Centralized volumetric MRI analysis allowed for sensitive measurement of PN volumes and can be incorporated in future trials with agents when shrinkage as drug effect is considered possible. Analysis of the effect of sirolimus on time to disease progression in progressive NF1 PNs is ongoing.

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