Clinical Protocol and Supporting Clinical Documentation

Protocol Title:

Medical Treatment of "high-risk" Neurofibromas in Patients with Type 1 Neurofibromatosis: A Clinical Trial of sequential medical therapies.

Phase: II

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1 ABSTRACT

Plexiform neurofibromas are commonly seen in patients with Neurofibromatosis type I. A significant number of these plexiform neurofibromas can result in severe disability from neurologic impingement, chronic pain, skeletal dysplasia, vascular complications, and/or organ dysfunction due to displacement or infiltration. Occasionally, these lesions are life-threatening due to the size or location of the tumor. Presently, there is no effective medical therapy to offer patients with "high-risk" plexiform neurofibromas. We have observed dramatic responses within the last two years in two patients with chronic "high-risk" plexiform neurofibromas. One patient, who had a large symptomatic pelvic "high-risk" plexiform neurofibroma, had complete resolution of symptoms along with approximately 50% tumor shrinkage with a treatment combination of naproxen and interferon alpha-2b. The second patient, who had a large cranio-facial "high-risk" plexiform neurofibroma that had already caused an ischemic stroke by compression of the carotid artery, experienced a complete radiographic response (regression of the plexiform tumor) with a combination of celecoxib and interferon alpha-2b together with vincristine and temozolomide. These preliminary findings were conducted without grant funding.

This clinical trial will further evaluate the effectiveness of these treatment combinations in patients with "high-risk" plexiform neurofibromas. Eligible patients will begin treatment with the combination of a cox-2 inhibitor (celecoxib) at a dose of 10 milligrams/kilogram/day (max 800 mg/day) and pegylated interferon alpha-2b at a dose of 1 microgram/kilogram/given weekly (CI). If the patients have at least a partial response after six months they may continue on the same treatment for up to two years. However, if the patient experiences less than a partial response, or has stable or progressive disease after six months of CI therapy, then vincristine (at a dose of 0.75 mg/m^2) and temozolomide (150 mg/m^2/day given 5 consecutive days each month) will be added to the celecoxib and interferon alpha-2b backbone (CITV). Response to the CITV therapy will be assessed after six months of treatment.

2 STUDY HYPOTHESES

The hypothesis to be tested is NF1 patients with "high-risk" plexiform neurofibromas that respond to treatment with celecoxib and pegylated interferon alpha-2b (CI) is significantly greater than 0—indicating an improved treatment response rate over traditional therapy.

The additional hypothesis to be tested is that "high-risk" plexiform neurofibromas who have demonstrated resistance to treatment with celecoxib and pegylated interferon alpha-2b (CI) will respond to the addition of temozolomide and vincristine (CITV), and this response rate will be significantly greater than 0.

2.1 Specific Aims

A. To define an algorithm for the medical treatment of "high-risk" plexiform neurofibromas using a sequential application of commercially available medications.

B. To evaluate tumor response based on objective evaluation of symptom assessment and tumor measurements.

C. To evaluate the toxicity of Celecoxib/Interferon alpha-2b and Celecoxib/Interferon alpha-2b/Temozolomide/Vincristine treatments in this selected population of young patients.

D. To specifically evaluate psychological toxicity of Celecoxib/Interferon alpha-2b and Celecoxib/Interferon alpha-2b/Temozolomide/Vincristine in this selected population of patients with NF-1

E. To improve the quality of life experienced by patients with "high-risk" plexiform neurofibromas by decreasing the pain and functional disability caused by these tumors.

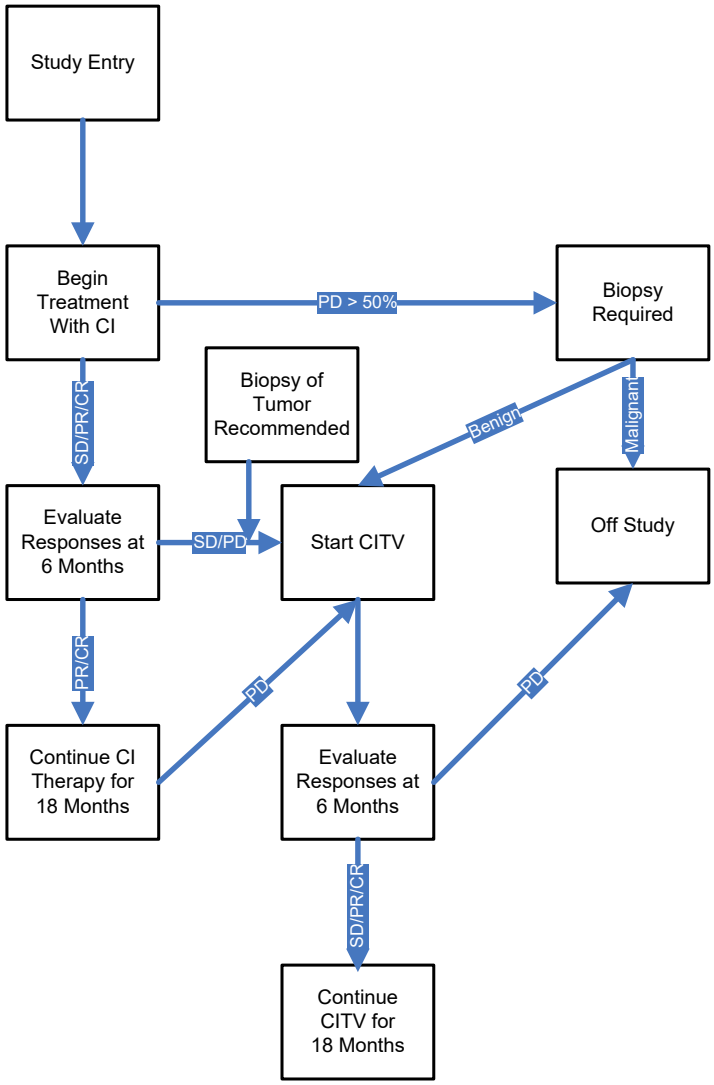
2.2 Study Design and Treatment Plan

This is a phase II single arm study with sequential treatments available by response where all participants begin therapy with a combination of celecoxib and interferon alpha-2b (CI, treatment-1). Response to CI therapy will be assessed at six months by clinical and radiographic evaluations. Those patients who have achieved a partial response (improvement in pain, improvement in functioning, or ≥50% reduction in tumor size) or complete response (resolution of pain, and normalization of functioning with a ≥ 90% reduction in tumor size) will continue with the same CI therapy for up-to two years on study.

Patients who have rapidly progressive disease (≥50% growth in tumor size) within the first six months of CI treatment are required to undergo tumor biopsy to evaluate for malignancy. If this biopsy is negative for malignancy then these patients may begin treatment with celecoxib/interferon alpha-2b/temozolomide/vincristine (CITV, treatment-2).

Patients who have not achieved at least a partial response to CI therapy after six months of treatment are also eligible to change to CITV treatment. For these patients a biopsy of the tumor is recommended but not required to verify the pathology of the tumor before advancing to CITV therapy.

Study Design



Legend

CI - Celocoxib + interferon alpha 2b
CITV - Celocoxib/Interferon alpha-2b/Temozolomide/Vincristine

PD - Progressive disease
SD - Stable disease
PR - Partial response (more than 50% shrinkage of the tumor)
CR - Complete response (more than 90% shrinkage of the tumor)

3 BACKGROUND

3.1 Plexiform Tumors in Neurofibromatosis Type 1 Patients

Neurofibromatosis type 1 (NF1) is a progressive genetic disorder with diverse cutaneous, neurologic, skeletal and neoplastic manifestations that affects 1 in 4000 persons [1]. While most of these persons are only mildly affected, some patients may experience severe compromise such as disabling disfigurement, neurologic impairment, or life-threatening compressions of vital structures. Three types of neurofibromas have been described in patients with NF1. a) Dermal/cutaneous: Soft, non-painful raised cutaneous lesions. b) Subcutaneous/solitary neurofibromas: Firm round masses that occur along the course of peripheral nerves, which are often painful and can compromise local structures as they enlarge. A variant of solitary neurofibromas may occur along the spine, which can extend into the spinal foramina and cause scoliosis or spinal cord compression. c) Plexiform neurofibromas: Soft poorly defined masses that often have the feeling of a bag of wet ropes. Plexiform neurofibromas are the most common cause of disfigurement in patients with NF1, and may be associated with life-threatening occlusion of the airway. In addition, plexiform neurofibromas are often associated with dysfunction of peripheral nerves with weakness, and painful neuropathy [2]. For the purposes of this study we consider any neurofibroma that is causing severe disfigurement (cosmetic or functional, i.e. spine), chronic pain, impairment of neurological function, or compression of vital structures to be "high-risk." Most of these "high-risk" neurofibromas are the plexiform type of neurofibroma.

3.2 Previous Treatments for Plexiform Tumors in NF1

Surgical resection is the only proven treatment for "high-risk" plexiform neurofibromas. Unfortunately, complete surgical resection is not possible for most patients with "high-risk" plexiform neurofibromas. Even when complete surgical resection is thought to be successful, half of the patients develop recurrent tumors after surgery. Partial surgical resection is not effective, but is sometimes used in patients facing imminent loss of function from these tumors. These limitations make surgical treatment of symptomatic neurofibromas on the face, spine and pelvis particularly difficult [1, 3, 4]. Therefore, the search for effective adjuvant medical therapies is warranted.

The medical treatment of "high-risk" plexiform neurofibromas has been disappointing. Ketotifen has been used to reduce the painful pruritis of solitary or plexiform neurofibromas, but they do not reduce the size of tumors [1]. Gupta and Cohen studied the use of thalidomide in patients with serious plexiform neurofibromas in patients with NF1. Four of the 12 patients studied did show a minor response to thalidomide with 2 of these 4 patients demonstrating radiographic improvement. Seven of 12 patients reported an improvement in neurofibroma related symptoms. However, the widespread use of thalidomide was limited by an increased risk of neuropathy and somnolence and the authors did not recommend thalidomide for future trials [3]. Interferon alpha (IFNα) has been the subject of a previous phase II clinical trial which has yet to be published. According to a recent review, 96% of patients receiving IFNα therapy were considered at least stable, with a few patients showing radiographic or symptomatic improvement [5].

3.3 Pathophysiology of Growth in Plexiform Tumors and Study Medications

The pathophysiology responsible for the development of plexiform neurofibromas in NF-1 patients is multi-factorial and only partially understood. It is known that plexiform neurofibromas are heterogeneous collections of cells including Schwann cells, fibroblasts, and mast cells [6]. While the Schwann cells are generally accepted as the neoplastic cell of origin, recent evidence suggests that the tumor stroma likely contributes to the proliferative state of these tumors. Stroma-derived elements including tumor growth factor beta (TGF- β) [7], matrix metalloproteinase(MMP)-9 [8] and vascular endothelial growth factor (VEGF) [9] are known to be elevated in plexiform neurofibromas, suggesting that they may serve as therapeutic targets. The initial agents selected for this trial were chosen in part for their known antagonism in these signaling pathways. Interferon has been shown to down-regulate both TGF-β mRNA production in fibroblasts derived from human hypertrophic scar tissue [10] and VEGF expression in human neuroendocrine tumor samples [11]. Cycloxygenase(COX)-2 inhibition (the inducible target enzyme of celecoxib) decreases both MMP and VEGF expression in animal models of breast, prostate, and colorectal cancers [12-15]. High-dose Celecoxib is now approved for use to reduce the incidence of adenomatous colorectal polyps in patients with Familial Adenomatous Polyposis.

Additional intrinsic cellular aberrations that result from the NF-1 defect justify the therapeutic use of both interferon and celecoxib. Neurofibromin normally inactivates the growth promoting pathway of RAS/mTOR [5]. NF-1 patients, being deficient in neurofibromin, have a functionally activated RAS/mTOR pathway. Interferon induced apoptosis has been shown to be mediated via the mTOR pathway, making neurofibromas the ideal target tumor type for interferon based therapies [16]. In addition to mTOR activation, oncogenic RAS mutations also up-regulate COX-2 expression which would further promote tumor growth and angiogenesis [17, 18] again making COX-2 inhibitors an intriguing therapy. Very little information exists on the interplay between interferons and COX-2. In vitro evidence suggests that interferon alpha induces COX-2 expression in vitro [19, 20], which may explain the partial benefit of single agent interferon therapy in NF-1 patients. A recent clinical trial of combination interferon alpha plus celecoxib therapy for metastatic renal cell carcinoma patients was completed and documented the safety of this combination [21].

3.4 Use of Chemotherapy for NF1

The use of conventional chemotherapy in NF-1 patients has been approached with much trepidation over concerns of mutagenesis. While it is true that NF-1 patients have a relative risk of malignancy that is approximately 4-6 fold higher than the general population [22], the use of chemotherapy (carboplatin) in NF-1 patients has never been shown to increase that risk further [23]. Temozolomide has shown benefit in the treatment of unresectable gliomas, tumor of neuronal origin, and was therefore chosen as the cytotoxic agent on this protocol [24]. The design of this protocol reserves the use of chemotherapy only for patients with persistent high-risk lesion despite the use of non-chemotherapeutic agents (celecoxib plus interferon). The combination of temozolomide and both interferon and celecoxib have been trialed separately in advanced melanoma patients and has shown some promise without discernable added toxicity [25, 26].

3.5 Preliminary Studies: Helen DeVos Children's Hospital Experience

We have had the opportunity to treat many patients with "high-risk" plexiform neurofibromas at Helen DeVos Children's Hospital (HDVCH), including patients who have participated in therapeutic clinical trials. While most of our experience using published therapy has been disappointing, there are two patients who within the last three years have had informative responses to treatment.

The first patient was referred to HDVCH for treatment of a large plexiform neurofibroma of the pelvis and lumbar spine which had caused chronic pain, leg weakness, and bladder dysfunction which was unresponsive to a third attempt at surgical treatment. This patient was started on a regimen of naproxen and Interferon alpha-2b and within six weeks this patient's chronic pain had resolved. Within 12 weeks the tumor was physically softer and measurably smaller on clinical examination. Along with these changes the patient's chronic leg weakness resolved, and bladder function become normal. After six months of therapy the mass was dramatically smaller on examination and measurable shrinkage by CT scan was noted. The patient was maintained on treatment with both medications for two years. Because of difficulty concentrating in school, the treatment has been reduced to naproxen alone. On recent follow-up examination the high-risk plexiform neurofibroma remains smaller than at diagnosis while using normal-dose naproxen.

The second patient is an infant that had a large disfiguring plexiform neurofibroma of the face and skull which was noted at birth. The growth of the tumor was controlled with interferon alpha-2b from 3 months of age but no tumor regression was noted on this therapy. At three years of age the patient developed a symptomatic optic glioma and the interferon was interrupted for 12 months while she received vincristine and carboplatin; (During this therapy the patients plexiform tumor was stable in size). Following this, the interferon was restarted but at five years of age the interferon alpha-2b was discontinued in order to participate in a therapeutic trial of thalidomide (Cleveland Clinic, Cleveland Ohio). After three months of treatment this patient's plexiform tumor was markedly larger, and the patient withdrew from the study medication. The patient continued to have slow growth of the tumor after re-starting interferon and so celecoxib was added. The patient showed a minor response to this therapy but had no further progression of her cranial-facial plexiform neurofibroma. Three years later (while continuing on treatment with celecoxib and interferon alpha-2b) the patient developed a progressive optic glioma and a symptomatic intrinsic brainstem tumor (never biopsied) which required additional therapy. Since the patient had previously shown rapid tumor growth when the interferon alpha-2b was discontinued for participation in the thalidomide trial, the celecoxib/interferon alpha-2b was continued while the patient received a combination of vincristine and temozolomide for treatment of these new tumors. The patient showed rapid shrinkage of her plexiform neurofibroma after the first treatment of celecoxcib/interferon alpha-2b/vincristine/temozolomide, and after three months of treatment had complete resolution of the cranial-facial plexiform neurofibroma. This patients' plexiform tumor continues to be in a clinical and radiographic remission after 24 months of treatment with celecoxib/interferon alpha-2b/vincristine/temozolomide.

3.6 NF1 and Pre-existing Neuropsychological Conditions

Children with NF1 most commonly demonstrate impairments in language, visual-spatial testing, attention and concentration [29]. Recently published studies find that between 52% and 60% of children with NF1 have significant learning impairments [27, 28]. Up to 81% of children with

NF1 can have moderate to severe impairment in one or more areas of cognitive functioning [29]. This information emphasizes the importance of obtaining a baseline evaluation of all children (and young adults) who participate in this study. It will be impossible to determine the true cognitive toxicity of interferon alpha 2b in this population without establishing a baseline for cognitive functioning for each patient.

4 INCLUSION CRITERIA

A. Patients must "high-risk" plexiform neurofibromas associated with a diagnosis of NF1 (see appendix III for diagnostic criteria for NF1). For the purposes of this study these tumors need to be disfiguring, causing chronic disability due to organ dysfunction or pain, or be in a life threatening location.

B. Patients must be 2-30 years old with a minimum bodyweight of 10 kilograms due to dosing restrictions associated with celecoxib. 10 adults (ages 18-30) and 10 pediatrics (ages 2-17) will be enrolled.

C. Patients and/or patient's guardian must voluntarily agree to provide informed consent for study participation.

D. Adequate renal function is required as measured by the serum creatinine. If the serum creatinine is higher than laboratory norms (indicating impaired renal function) a 24 hour urinary creatinine clearance will be ordered. If the creatinine clearance is less than 40ml/min/1.73 m^2, these patients are excluded from study participation.

E. Volunteer Screening Procedures: At the direction of the study investigators, patients will be screened by the Research Coordinator for potential participation in the study following all inclusion/exclusion criteria.

5 EXCLUSION CRITERIA

A. Patients who have a previously untreated active optic glioma are not considered eligible for this study since other medically accepted therapy is available for first-line treatment of this tumor.

B. Patients who have a history of any previous allergy to study medications (Celecoxib, Interferon alpha-2b, Temozolomide, Vincristine) are excluded from study participation.

C. Patients with a known history of ischemic vascular disease will be excluded from study participation. Both interferon and celecoxib can aggravate ischemic vascular disease. Study participation by an individual with known peripheral vascular disease may place the participant at undue risk of renal insufficiency, cardiovascular disease, and/or stroke.

D. Female patients who are pregnant are excluded from study participation, these study medications may be damaging to a developing fetus and should not be used in women who are pregnant.

E. Women who are breast feeding are excluded from this study.

F. Patients who are identified to have significant depression or other psychological disturbance that require medical intervention are excluded from this study.

5.1 Criteria for Removal from Study Participation

A. Patients who experience a rapid growth of their tumor during the first six months of treatment are eligible to remain on study if they have a tumor biopsy that pathologically confirms a plexiform neurofibroma and excludes a malignant tumor phenotype.

B. Pathologic diagnosis of malignancy will result in removal from study participation.

C. Patients who are identified to have significant depression or other psychological disturbance that require medical intervention will be removed from study and discontinue all study medications. These patients will then be referred to their primary care physician for further treatment.

D. Patients will be asked to bring any unused study medications with them to each visit. Patients who consistently have less than an 80% compliance rate with study medications (by direct counting of injections and or pills) will be removed from study. However, patients who receive less than 80% of the specified dose of study medications due to toxicity mandated dose reductions will remain study eligible.

E. Women who become pregnant during study therapy will be removed from study participation, and therapy will be discontinued since any of the study prescribed medications may be dangerous to a developing fetus.

5.2 Inclusion of Women and Minorities: Women and minorities who fit all inclusion/exclusion criteria will be included in this study.

6 OBSERVATIONS

Along with the following, additional tests/procedures/evaluations may be performed as deemed necessary for patient care by the study investigator.

6.1 Pre-Treatment Evaluation

A. Initial physical examination with measurement of weight, blood pressure, clinical tumor measurements, and evaluation of activity using the Karnofsky Performance Scale (for patients >16 years old) or the Lansky Play Performance Scale (for patients ≤16 years old) scale assessment

B. Photographic documentation of tumor appearance will be requested and included in the study record following patient/guardian consent. These images will be available only to study personnel.

Photographic records of tumors at diagnosis and throughout the study will be kept on a separate electronic recording device organized by unique study number (not by name). These records will be retained for an indefinite length of time. This information will not be released to other institutions or physicians without the expressed written consent of the patient or patient guardian. Written consent will be obtained from the patient or patient guardian prior to submission of these images for later publication.

C. Complete blood count with differential and chemistry profile that includes electrolytes, Ca, PO_4, BUN, Cr, Bilirubin, ALT, and AST.

D. Urine pregnancy test for all female patients of childbearing potential must be negative at study entry.

E. Psychological evaluation by a licensed psychologist. See Appendix 4 for detailed information regarding evaluation.

F. MRI studies will be ordered locally and must be completed within 8 weeks of study participation. All MRI images for this study will be sent via data storage device to Dr. Brigitte Widemann at the National Institutes of Health (a collaborating physician at the NIH) for 3-dimensional volumetric measurements after de-identification by Spectrum Health radiological services.

6.2 Evaluations During Protocol Therapy

A. Monthly physical exams (+/- 7 days) for the first 3 months to monitor toxicity, and every three months (+/- 14 days) for the duration of study participation. Attention to psychological side effects will be a part of each physical examination.

B. Photographic documentation of tumor appearance will be requested every three months (+/- 14 days) of treatment, and included in the study record following patient/guardian consent.

C. Monthly (+/- 7 days) CBC and chemistry profile that includes electrolytes, Ca, PO_4, BUN, Cr, Bilirubin, ALT, and AST for the first six months. If the patient continues Treatment Arm 1, then these laboratory studies can be evaluated every 3 months (+/- 14 days) at the discretion of the study physician.

D. For patients who proceed on to Treatment Arm 2, monthly (+/- 7 days) CBC and chemistry profile that includes electrolytes, Ca, PO_4, BUN, Cr, Bilirubin, ALT, and AST will be evaluated for the duration of trial therapy.

E. Psychological evaluation by a licensed psychologist will occur at 3 months, 12 months, and 24 months, (+/- 30 days), of study participation with more frequent evaluations as clinically indicated (see Appendix 4 for detailed information regarding evaluation). Study participants and family members will be encouraged to call study personnel with concerns about psychological functioning.

F. MRI studies will be ordered locally after 6, 12, and 24 months, (+/- 30 days), of study participation to evaluate tumor response, these images will then be sent to Dr. Brigitte Widemann at the National Institutes of Health (a collaborating physician at the NIH) for 3-dimensional volumetric measurements. Additional studies can be ordered as deemed necessary for patient care by the study physician. Any studies done for other purposes will also be evaluated for tumor response information.

G. Female patients of childbearing age must have a negative urine pregnancy test before participating and participants must agree to disclose potential pregnancy as soon as suspected to the study physician or nurse.

H. Female participants of childbearing potential who proceed to treatment arm 2 will require a urine pregnancy screening test with each monthly visit to avoid fetal injury from chemotherapy. If a pregnancy is confirmed then the patient will be removed from study participation.

I. Patients who are found to have active psychosis or depression that may be worsened by the use of interferon are excluded from study participation.

6.3 End of Therapy Evaluations

A. Patients will undergo detailed physical examination with measurement of weight, blood pressure, clinical tumor measurements, and evaluation of activity using the Karnofsky Performance Scale (for patients >16 years old) or the Lansky Play Performance Scale (for patients ≤16 years old) scale assessment.

B. Photographic documentation of tumor appearance will be requested and included in the study record following patient/guardian consent.

C. Complete blood count with differential and chemistry profile that includes electrolytes, Ca, PO_4, BUN, Cr, Bilirubin, ALT, and AST.

D. Psychological evaluation by a licensed psychologist. See Appendix 4 for detailed information regarding evaluation.

E. MRI studies will be ordered locally, these images will then be sent to Dr. Brigitte Widemann at the National Institutes of Health (a collaborating physician at the NIH) for 3-dimensional volumetric measurements.

6.4 Off Therapy Follow-up of Responding Patients

Patients who respond to this therapy will be asked to return for follow-up evaluations every three months (+/- 14 days) for the first year off therapy and every six months (+/- 30 days) for the next three years off therapy to determine the duration of response. Evaluations will include:

A. Patients will undergo detailed physical examination with measurement of weight, blood pressure, clinical tumor measurements, and evaluation of activity using the Karnofsky Performance Scale (for patients >16 years old) or the Lansky Play Performance Scale (for patients ≤16 years old) scale assessment.

B. Photographic documentation of tumor appearance will be requested and included in the study record following patient/guardian consent.

C. MRI studies may be ordered to evaluate tumor response, these images will then be sent to Dr. Brigitte Widemann at the National Institutes of Health (a collaborating physician at the NIH) for 3-dimensional volumetric measurements. Any studies done for other purposes will also be evaluated for tumor response information.

D. Psychological evaluation by a licensed psychologist, if the end of therapy evaluation showed detrimental changes for the on-study evaluation. See Appendix 4 for detailed information regarding evaluation.

6.5 Response Criteria

A. PD - Progressive disease, with an increase of at least 20% over previous tumor evaluations

B. SD - Stable disease, tumors that measure between 20% larger and 50% smaller than previous measurement with no improvement in tumor related symptoms and daily functioning.

C. PR - Partial response, 50-90% shrinkage of the tumor,

D. PR-S - Less than 50% shrinkage of the tumor with marked improvement in tumor related symptoms and daily functioning

E. CR - Complete response, more than 90% shrinkage of the tumor with associated resolution of tumor associated symptoms and disability not caused by abnormal bone growth. Patients with persistent symptoms resulting from preceding orthopedic or neurologic damage will not be considered as persisting tumor-related symptoms and may qualify at complete responders.

7 STUDY TREATMENT

7.1 Treatment Duration:
Each treatment arm will be given for a minimum of six months, presuming the patient has not experienced progressive disease, before advancing to the next arm. Total duration of therapy on study is two years for any individual treatment plan (i.e. treatment-1 or treatment-2).

7.2 Treatment Arm 1

A. Celecoxib 10 mg/kg/day given by mouth (once or twice daily). See Appendix 2 for dosage table by weight.

B. Pegylated interferon alpha-2b at a dose of 1 microgram(mcg)/kg of body weight given as a subcutaneous injection weekly. This dose was well tolerated by 26/28 patients in a previous trial [30]. To reduce the toxic side effects associated with beginning interferon therapy, the patients will begin with a dose of 0.3 mcg/kg, then escalate to 0.6 mcg/kg at week two, and 1 mcg/kg at week 3. Diphenhydramine at 1 mg/kg orally is recommended one time before and tid for 24 hours after the first interferon dose. Pre-medication with diphenhydramine at 1 mg/kg orally is recommended before each dose of interferon until drug tolerance is established.

1. If a patient experiences fever >40^{o}C, rigors, or appetite suppression with weight loss, then the dose of interferon will be reduced to the previous dosage until the identified symptoms improve before resuming dosage escalation.

2. *Warning*: Interferons, including interferon alfa-2b, can cause or aggravate fatal or life-threatening neuropsychiatric (including suicidal ideation and attempts), autoimmune, ischemic, and infectious disorders. Patients will be monitored closely with monthly clinical and laboratory evaluations. Patients with persistently severe or worsening signs or symptoms of these conditions will be

withdrawn from therapy. In many but not all cases these disorders resolve after stopping interferon alfa-2b therapy.

C. If a study patient has progressive disease or no response to treatment 1 after six months, he/she will proceed to treatment 2. Patients who experience a rapid growth of tumor on treatment 1 must first undergo a biopsy of this tumor to determine the pathologic phenotype of this tumor. Patients with a malignant phenotype will be removed from study participation and will be referred for appropriate medical treatment.

7.3 Treatment Arm 2

A. Continue treatment arm 1 medications and add:

B. Vincristine (Oncovin®) 0.75 mg/m^2/dose or 0.025 mg/kg/dose if the patient weighs <15kg (maximum dose 2mg) to be given intravenously weekly x 4 doses then every month with temozolomide [vincristine dose is 50% of normal because of concurrent interferon]. The dose of vincristine will be adjusted for grade 3 or 4 toxicity. See page 20 (section 9.4).

C. Temozolomide (Temodar®) (150 mg/m^2/day) for 5 consecutive days given by mouth every month. Fractionate Temodar® into 2-3 daily doses with appropriate antiemetic coverage before each dose.

7.4 Dose Adjustments

A. **Hepatic impairment**: discontinue therapy if serum glutamic oxaloacetic transaminase exceeds 5 times the upper limit of normal.

B. **Hematologic**: WBC count less than 1.5 x 10(9) /L; granulocyte count less than 0.75 x 10(9) /L; platelet count less than 50 x 10(9) /L) reduce dose of temozolomide by 50% and escalate to full dose as tolerated. Peg- interferon alpha 2a can also cause hematologic toxicity, if the patient is not receiving temozolomide, the peg- interferon alpha 2a dose will be reduced by 50% and escalate to full dose as tolerated.

C. **Hematologic**: WBC count less than 1 x 10(9) /L; granulocyte count less than 0.5 x 10(9) /L; platelet count less than 25 x 10(9) /L) hold and restart temozolomide (or peg-interferon alpha 2a) at less than 50% of previous dose after hematologic recovery and escalate to full dose as tolerated.

Special Circumstances: In the event that a patient with a pre-existing hematologic condition that is associated with cytopenias (neutropenia, anemia or thrombocytopenia) qualifies for study participation, then the interpretation of these hematologic toxicity guidelines may be cautiously adjusted. Specifically, patients who are receiving celocoxib/interferon (CI) may receive supportive care utilizing growth factors (g-CSF [Neupogen], erythropoietin), prophylactic antibiotics, or immunoglobulin (ie IVIG for immune thrombocytopenia Purpura) to reduce the complications associated with these cytopenias while continuing to receive full doses of study medications. Steroids should not be used in that they may also affect tumor response. However, should additional complications from these cytopenias occur (fever with neutropenia, clinical infections, or bleeding with thrombocytopenia), then adjustment of study medications to assure patient safety must take place.

Patients who are receiving celocoxib/interferon/temozolomide/vincristine (CITV) do not qualify for this 'exception' and must follow the listed dose adjustment guidelines for cytopenias.

D. **Renal impairment**: discontinue therapy if the serum creatinine exceeds 2 mg/dL

E. **Severe adverse reactions**: temporarily discontinue therapy if severe adverse reactions develop. When the patient has recovered, interferon may be restarted at 50% of the previous dosage and escalated to full dose as tolerated at the discretion of the study physician.

F. **Psychological**: Interferons, including interferon alfa-2b, can cause or aggravate life-threatening neuropsychiatric disorders. Patients will be monitored closely with monthly clinical evaluations. Patients with persistently severe or worsening signs or symptoms of these conditions will be withdrawn from therapy. In many but not all cases these disorders resolve after stopping interferon alfa-2b therapy. Patients with milder exacerbations of psychological dysfunction will be referred to a psychiatrist for consideration of treatments to control the symptoms without completely discontinuing study medications.

7.5 Adverse Events

A. Definition of an adverse event: For the purposes of this study, an adverse event is defined as any inter-current illness which produces an adverse change from pretreatment baseline during the course of the study, whether the adverse event is considered related to the study treatment or not. Adverse events that are graded as grade 3 or 4 by the common Terminology Criteria Version 3.0 for adverse events as published by the U.S. Department of Health and Human Services will be recorded.

B. Definition of a serious adverse event: Serious adverse events (SAE) include any event resulting in death, any life-threatening experience, an event that causes or prolongs hospitalization, any event that causes persistent or significant disability or incapacity, or events that require medical or surgical intervention to prevent the above.

C. Adverse event reporting: At each contact with the subject, the investigator will seek information on adverse events by specific questioning and as needed examination. Information on adverse events including onset date, duration, severity, relationship to the investigational agent, action taken with respect to the investigational agent and outcome will be recorded in the source document and also in the appropriate case report form (CRF).

The clinical course of each event will be followed until resolution, stabilization, or until it is determined that the study treatment is not the cause of the adverse event. Serious adverse events at the end of the study period will be followed to determine the final outcome. Any serious adverse event that occurs after the study period that is considered to be possibly related to the study treatment will be recorded and reported immediately.

All unexpected and related adverse events will be reported to the Spectrum Health IRB (Institutional Review Board), per Spectrum Health IRB policies.

D. Data and safety monitoring: A multidisciplinary committee (composed of principal investigator and/or sub-investiators, pharmacist, nurses, and research study staff) will evaluate this study for the purpose of data and safety monitoring. The committee will evaluate the need for amendments, revisions, and/or study discontinuation at least quarterly during the time of active patient participation. More frequent meetings will be arranged as needed for toxicity monitoring.

8 STATISTICAL CONSIDERATIONS

The endpoint of this study is response to therapy which will be established clinically as defined elsewhere in this proposal. As long as patients show measurable response based on performance criteria (using Lansky or Karnofsky Performance Status Scales, see Appendix 1) or tumor evaluation they are considered a responder. The simple design of this study allows for a binary statistical evaluation of response, where a response is considered anything more than 0 (which is considered to be stable or progressive disease).

The unknown factor in determining sample size is the number of patients who will respond to treatment arm 1 with celecoxib/interferon alpha-2b("CI"). If most study participants show a response to treatment arm 1, then treatment arm 2 may not have an adequate number of participants to determine response rates (even given this simple design). We anticipate a response rate approaching 50% to treatment arm 1. This estimate a total study size of 20 patients is required to provide up to 10 patients eligibility to proceed to celecoxib/interferon alph-2b/temozolomide/vincristine (CITV) treatment (i.e., Treatment Arm 2). However, a significant number of patients may withdraw from study participation due to toxicity. Thus we recommend accruing 10 patients in each treatment arm with a total sample size of 20 patients so that a minimum of 10 patients will be available for evaluation in treatment arm 2.

This phase II clinical trial aims to establish the efficacy of a novel treatment for NF1. Patients receiving this treatment will be in treatment arm 2, having already failed non-chemotherapy based therapy in treatment arm I. Therefore, the alternative hypothesis to be tested is that response to treatment arm 2 is significantly greater than 0—indicating improvement in this group of patients over non-chemotherapy based therapy. The corresponding null hypothesis being that the response rate in treatment arm 2 is 0. Under the assumption of independence, only a handful of patients should be necessary to reject the null hypothesis (approximately 5 if a response rate of 20% is expected in arm 2). However, since the samples are not independent, this sample size must be increased somewhat. Since a response rate of approximately 50% is expected in arm 1, approximately 10 patients will be available for arm 2, which should be adequate to demonstrate a response rate > 0 in arm 2 even after adjustment for the non-independence of the samples.

9 DRUG INFORMATION

9.1 Celecoxib (Celebrex®) revised Aug 2002

Source and Pharmacology: Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) that is a selective inhibitor of COX-2 (cyclooxygenase-2). Cyclooxygenases 1 and 2 are enzymes that are involved with conversion of arachidonic acid to prostaglandins. COX-1 is present normally in much of the body and is particularly involved in platelet aggregation and protective

mechanisms of the gut and kidney. COX-2 is formed in higher concentrations in response to inflammation and injury. Selective inhibitors of COX-2 have analgesic, anti-inflammatory, and antipyretic effects, with less frequent toxicity to the gut and kidneys, and little-to-no inhibition of platelet function. Prostaglandins that are produced by COX 1 and 2 appear to be involved with angiogenesis, and celecoxib has been shown to have anti-angiogenic effects. At high doses, it has been shown to reduce the formation of polyps in patients with familial adenomatous polyposis. Celecoxib is metabolized to inactive metabolites primarily by P-450 2C9. It appears that some drugs that stimulate or inhibit P-450 2C9 enzymes can increase or decrease the clearance of celecoxib. Celecoxib may inhibit P-450 2D6 and reduce the clearance of drugs metabolized primarily by that route. Celecoxib is approximately 97% bound to plasma proteins. The elimination half-life of celecoxib is approximately 11 hours.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Dyspepsia, may mask fever in neutropenic patients (L)		Hypersensitivity reactions (especially with history of sulfa or aspirin hypersensitivity, or asthma triad)
Prompt: Within 2-3 weeks, prior to the next course			Fluid retention (L), edema, acute renal failure (L), liver dysfunction
Delayed: Any time later during therapy, excluding the above conditions			GI bleeds, peptic ulcer disease Cardiovascular disease, known or risk factors for; increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke
Late: Any time after completion of treatment			

(L) Toxicity may also occur later.

Supplier: Commercially available. See package insert for more detailed information.

9.2 Peg-Interferon alpha-2b (PEG-Intron™) Revised May 2007

Source and Pharmacology: Interferon alpha is a protein produced by recombinant DNA techniques. Intron A and Roferon A are obtained from strains of E. Coli bearing a genetically-engineered plasmid containing either interferon alpha 2b or interferon alpha 2a gene, respectively, from human leukocytes. Their structures differ by one amino acid at the 26 position. Alpha-IFN

has been shown to have antiviral, immunomodulatory, and antiproliferative effects both in vitro and in vivo. Interferons bind to specific membrane receptors on the cell surface and induce several enzymes which result in decreased protein synthesis and DNA synthesis by acting on transcriptional and translational processes. Other enzymes are induced by IFN and contribute to a) the inhibition of virus replication in virus-infected cells, b) suppression of cell proliferation, and c) such immunomodulation activities as enhancement of phagocytic activity of macrophages and augmentation of the specific cytotoxicity of lymphocytes for target cells.

Following a single subcutaneous (SC) dose of PEG-Intron, the mean absorption half-life (t ½ k_a) was 4.6 hours. Maximal serum concentrations (C_{max}) occur between 15-44 hours post-dose, and are sustained for up to 48-72 hours. The C_{max} and AUC measurements of PEG-Intron increase in a dose-related manner. After multiple dosing, there is an increase in bioavailability of PEG-Intron. Week 48 mean trough concentrations (320 pg/mL; range 0, 2960) are approximately 3-fold higher than Week 4 mean trough concentrations (94 pg/mL; range 0, 416). The mean PEG-Intron elimination half-life is approximately 40 hours (range 22 to 60 hours) in patients with HCV infection. The apparent clearance of PEG-Intron is estimated to be approximately 22.0 mL/hr·kg. Renal elimination accounts for 30% of the clearance. Single dose peginterferon alfa-2b pharmacokinetics following a subcutaneous 1.0 µg/kg dose suggest the clearance of peginterferon alfa-2b is reduced by approximately half in patients with impaired renal function (creatinine clearance <50 mL/minute). Pegylation of interferon alfa-2b produces a product (PEG-Intron) whose clearance is lower than that of non-pegylated interferon alfa-2b. When compared to INTRON A, PEG-Intron (1.0 µg/kg) has approximately a seven-fold lower mean apparent clearance and a five-fold greater mean half-life permitting a reduced dosing frequency. At effective therapeutic doses, PEG-Intron has approximately ten-fold greater C_{max} and 50-fold greater AUC than interferon alfa-2b.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Fever, fatigue, headache, site reaction, rigors, myalgia, myelosuppression	anorexia, nausea, arthralgia, diarrhea (L)	Vomiting, chills, stomatitis, somnolence (L), psychosis (L), elevated transaminases, peripheral neuropathy (L), sinus tachyrhythmias, hypocalcemia, pancreatitis, hyperkalemia, anaphylaxis, dyspnea, hypotension
Prompt: Within 2-3 weeks, prior to the next course		depression	Rash, dizziness, impotence, thyroid function changes, cardiovascular events
Delayed: Any time later during therapy, excluding the above conditions			Alopecia, menstrual disorder, colitis, ophthalmologic disorders
Late: Any time after completion of treatment			

(L) Toxicity may also occur later.

Supplier: Commercially available. See package insert for more detailed information.

Warning: Interferons, including interferon alfa-2b, can cause or aggravate fatal or life-threatening neuropsychiatric (including suicidal ideation and attempts), autoimmune, ischemic,

and infectious disorders. Patients will be monitored closely with monthly clinical and laboratory evaluations. Patients with persistently severe or worsening signs or symptoms of these conditions will be withdrawn from therapy. In many but not all cases these disorders resolve after stopping interferon alfa-2b therapy.

9.3 Temozolomide (Temodar™) NSC # 362856 Revised: Aug 2001

Source and Pharmacology: An orally administered alkylating agent, a second generation imadazotetrazine. A prodrug of MTIC, temozolamide spontaneously decomposes to MTIC at physiologic pH. Exerts its effect by cross-linking DNA. This is likely a site specific alkylation at the O^6-position of guanine with some effect at the N7 position. Temozolamide reaches its peak concentration in 1 hour. Food reduces the rate and extent of absorption. It has an elimination half-life of 1.13hr (intraperitoneally) and 1.29hxr (orally) with an oral bioavailability of 0.98. Total apparent body clearance is 100ml/min/m^2 and plasma elimination half-life is ~100 minutes.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Anorexia, nausea, vomiting, abdominal pain, diarrhea, constipation, headache, rash, itching, urinary frequency and/or infection	Convulsions, hemiparesis, dizziness, ataxia, confusion, dysphagia, anxiety, thrombo-embolism (L)
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression	Mucositis, lethargy, peripheral edema	Amnesia, insomnia, depression, myalgia, diplopia, visual changes
Delayed: Anytime later during therapy, excluding the above conditions		Alopecia, hepatotoxicity	
Late: Anytime after completion of therapy			Secondary tumors or cancer

(L) Toxicity may also occur later.

Supplier: Commercially available. See package insert for more detailed information.

9.4 Vincristine Sulfate (VCR, Oncovin) NSC #067574 Revised: Nov. 2003
Approved roadmap abbreviation: VCR

Source and Pharmacology: Vincristine is an alkaloid isolated from Vinca rosea (periwinkle). It binds to tubulin, disrupting mircotubules and inducing metaphase arrest. Its serum decay pattern is triphasic, with initial, middle and terminal half-lives of 5 minutes, 1.3 hours, and greater than 24 hours , respectively. It is excreted in the bile and feces. There is poor CSF penetration.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Local ulceration if extravasated	Jaw pain	
Prompt: Within 2-3 weeks, prior to the next course	Alopecia	Weakness, constipation, abdominal pain	Paralytic ileus, ptosis, diplopia, night blindness, vocal cord paralysis, myelosuppression, SIADH, seizure
Delayed: Any time later during therapy, excluding the above conditions	Loss of deep tendon reflexes	Numbness, tingling and clumsiness	Veno-occlusive disease, blindness, optic atrophy
Late: Any time after the completion of treatment			
Unknown Frequency and Timing: **Fetal and teratogenic toxicities			

**Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.

Formulation and Stability: Available in solutions of 1mg/1ml in 1, 2, or 5ml vials. Refrigerate and protect from light. Once opened, it should be refrigerated and used within 10 days. Note: The drug is light-sensitive.

Guidelines for Administration: IV push over ≤1 minute. Special Precautions: Avoid extravasation. Decrease dose for infants (e.g., less than 10 kg, divide m^2 dose by 30, multiply by weight in kg. Alternatively, give 50% of calculated dose). Precaution: Concomitant radiation therapy to the liver may enhance toxicity. Precaution: When dispensing vincristine in other than the original container, it is imperative that it be packaged in the provided overwrap which bears the following statement: "Do not remove covering until moment of injection. Fatal if given intrathecally. For intravenous use only."

Supplier: Commercially available. See package insert for further information.

9.4.1 Dose Modification Guidelines for Vincristine:

Definition of Neuropathy

A. Severe neuropathic pain (grade 3 or greater): Hold dose(s). When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated.

NOTE: neuropathic pain can be not only severe but difficult to treat. However, because vincristine is an important component of curative therapy and the majority of neuropathies are ultimately reversible, vincristinetherapy may be given at full dose at investigator discretion. Severe peripheral neuropathies, with or without a positive family history might suggest the need for a molecular diagnostic evaluation to rule out Charcot Marie Tooth Disease (CMT), Type 1A or Hereditary neuropathy with liability to pressure palsies. Drugs such as gabapentin may be of value.

B. Vocal Cord paralysis: Hold dose(s). When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated.

C. Foot Drop, paresis: Should be grade 3 to consider holding or decreasing dose. These toxicities are largely reversible but over months to years. Accordingly, holding doses of vincristine and/or lowering the dose may not result in rapid resolution of symptoms and may compromise cure. See above for comment on CMT. Physical therapy may be beneficial to maintain range of motion and provide AFO's and other forms of support. Drugs such as gabapentin may be of value.

D. Jaw pain: Treat with analgesics; do not modify vincristine dose.

E. Constipation or ileus (> Grade 3) or typhlitis: Hold dose(s); institute aggressive regimen to treat constipation if present. When symptoms abate resume at 50% dose and escalate to full dose as tolerated.

9.4.2 Grading of Sensory Neuropathy (from Balis):

Grade 1: Paresthesias, pain, or numbness that do not require treatment or interfere with extremity function.

Grade 2: Paresthesias, pain, or numbness that are controlled by non-narcotic medications (without causing loss of function), or alteration of fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait, without abrogating ability to perform these tasks.

Grade 3: Paresthesias or pain that are controlled by narcotics, or interfere with extremity function (gait, fine motor skills as outlined above), or quality of life (loss of sleep, ability to perform normal activities severely impaired).

Grade 4: Complete loss of sensation, or pain that is not controlled by narcotics.

9.4.3 Grading of Motor Neuropathy (from Balis):

Grade 1: Subjective weakness, but no deficits detected on neurological exam, other than abnormal deep- tendon reflexes.

Grade 2: Weakness that alters fine motor skills (buttoning shirt, coloring, writing or drawing, using eating utensils) or gait without abrogating ability to perform these tasks.

Grade 3: Unable to perform fine motor tasks (buttoning shirt, coloring, writing or drawing, using eating utensils) or unable to ambulate without assistance.

Grade 4: Paralysis.

9.4.4 Hyperbilirubinemia

Direct Bili Dose reduction

< 3.1 mg/dl	Full dose
3.1- 5.0 mg/dl	50%
5.1-6.0 mg/dl	75%
6.0 mg/dl	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

9.4.5 Extravasations:

In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Suggestions below may be helpful but none are considered definitive:

A. Stop infusion, aspirate drug and blood if possible, remove needle

B. Apply warm compress immediately for 1 hour then rotate on/off every 15 minutes for 24 hours.

C. Hyaluronidase 150 units/ml reconstituted with NS-inject 1ml for each 1 ml of drug extravagated or

D. Treat with cold compresses, dilute thru infiltration of 5 mL of an 8.4% solution of sodium bicarbonate and/or local injection of hydrocortisone.

10 SUBJECT STIPENDS OR PAYMENTS

Study subjects will receive a gift card following the completion of study visits to reimburse for expenses that are a result of study participation. Subjects will be compensated as follows:

Subject lives less than 75 miles from the hospital: $25 gift card
Subject lives more than 75 miles from the hospital: $50 gift card
Subject lives more than 300 miles from the hospital: $100 gift card

As multiple tests or evaluations may be required for a particular study visit, subjects will receive a maximum of one gift card per month.

11 DATA HANDLING AND RECORD KEEPING

11.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Signed informed consent for study participation will include a HIPAA authorization.

In the event that a subject revokes authorization to collect or use protected health information (PHI), the investigator will retain the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect vital status at the end of their scheduled study period. Any study information sent out of Spectrum Health will be de-identified.

11.2 Source Documentation and Case Report Forms

Source data is all information, original records of clinical finding, observations, or other activities in a clinical trial, necessary for the reconstruction and evaluation of the trial. Source data are contained in the source documents, and will be recorded within 24 hours of patient encounter.

A study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or 'white-out' errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date the entry.

11.3 Data Storage

All study records will be kept in a restricted access facility. Hard copy records will be stored in a locked room or filing cabinet. Data will be retained within a Spectrum Health facility for a duration according to Spectrum Health policy requirements and applicable regulations.

11.4 Data Monitoring

The investigators will permit study-related monitoring, audits, and inspections by the Spectrum Health IRB, appropriate government regulatory bodies, and Spectrum Health research compliance and quality assurance groups of all study related documents.

12 REFERENCES CITED:

1. Eichenfield, L.F., et al., *Guidelines of care for neurofibromatosis type 1. American Academy of Dermatology Guidelines/Outcomes Committee.* J Am Acad Dermatol., 1997. **37**(4): p. 625-30.
2. Roos, K.L. and D.W. Dunn, *Neurofibromatoses.* CA Cancer J Clin., 1992. **42**(4): p. 241-54.
3. Gupta, A., et al., *Phase I study of thalidomide for the treatment of plexiform neurofibroma in neurofibromatosis 1.* Neurology., 2003. **60**(1): p. 130-2.
4. Needle, M.N., et al., *Prognostic signs in the surgical management of plexiform neurofibroma: the Children's Hospital of Philadelphia experience, 1974-1994.* J Pediatr., 1997. **131**(5): p. 678-82.
5. Packer, R.J., et al., *Plexiform neurofibromas in NF1: toward biologic-based therapy.* Neurology., 2002. **58**(10): p. 1461-70.
6. Le, L.Q. and L.F. Parada, *Tumor microenvironment and neurofibromatosis type I: connecting the GAPs.* Oncogene, 2007. **12**: p. 12.
7. Yang, F.C., et al., *Nf1+/- mast cells induce neurofibroma like phenotypes through secreted TGF-beta signaling.* Hum Mol Genet., 2006. **15**(16): p. 2421-37. Epub 2006 Jul 11.
8. Levy, P., et al., *Molecular profiles of neurofibromatosis type 1-associated plexiform neurofibromas: identification of a gene expression signature of poor prognosis.* Clin Cancer Res., 2004. **10**(11): p. 3763-71.
9. Kawachi, Y., et al., *Expression of angiogenic factors in neurofibromas.* Exp Dermatol., 2003. **12**(4): p. 412-7.
10. Tredget, E.E., et al., *Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma in vitro and in vivo.* J Interferon Cytokine Res., 2000. **20**(2): p. 143-51.
11. Rosewicz, S., et al., *Interferon-alpha: regulatory effects on cell cycle and angiogenesis.* Neuroendocrinology., 2004. **80**(Suppl 1): p. 85-93.
12. Yoshida, S., et al., *COX-2/VEGF-dependent facilitation of tumor-associated angiogenesis and tumor growth in vivo.* Lab Invest., 2003. **83**(10): p. 1385-94.
13. Larkins, T.L., et al., *Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression.* BMC Cancer., 2006. **6**: p. 181.
14. Yao, M., et al., *Cyclooxygenase-2 selective inhibition with NS-398 suppresses proliferation and invasiveness and delays liver metastasis in colorectal cancer.* Br J Cancer., 2004. **90**(3): p. 712-9.
15. Attiga, F.A., et al., *Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases.* Cancer Res., 2000. **60**(16): p. 4629-37.
16. Thyrell, L., et al., *Interferon alpha-induced apoptosis in tumor cells is mediated through the phosphoinositide 3-kinase/mammalian target of rapamycin signaling pathway.* J Biol Chem., 2004. **279**(23): p. 24152-62. Epub 2004 Mar 31.
17. Hoang, B., et al., *Oncogenic RAS mutations in myeloma cells selectively induce cox-2 expression, which participates in enhanced adhesion to fibronectin and chemoresistance.* Blood., 2006. **107**(11): p. 4484-90. Epub 2006 Feb 23.

18. Sheng, H., J. Shao, and R.N. Dubois, *K-Ras-mediated increase in cyclooxygenase 2 mRNA stability involves activation of the protein kinase B1.* Cancer Res., 2001. **61**(6): p. 2670-5.
19. Lee, J., et al., *Interferon-alpha resistance can be reversed by inhibition of IFN-alpha-induced COX-2 expression potentially via STAT1 activation in A549 cells.* Oncol Rep., 2006. **15**(6): p. 1541-9.
20. Bostrom, P.J., et al., *Interferon-alpha inhibits cyclooxygenase-1 and stimulates cyclooxygenase-2 expression in bladder cancer cells in vitro.* Urol Res., 2001. **29**(1): p. 20-4.
21. Rini, B.I., et al., *Maximal COX-2 immunostaining and clinical response to celecoxib and interferon alpha therapy in metastatic renal cell carcinoma.* Cancer., 2006. **106**(3): p. 566-75.
22. Korf, B.R., *Malignancy in neurofibromatosis type 1.* Oncologist., 2000. **5**(6): p. 477-85.
23. Listernick, R., et al., *Carboplatin therapy for optic pathway tumors in children with neurofibromatosis type-1.* J Neurooncol., 1999. **45**(2): p. 185-90.
24. Gururangan, S., et al., *Temozolomide in children with progressive low-grade glioma.* Neuro-oncol., 2007. **9**(2): p. 161-8. Epub 2007 Mar 8.
25. Garcia, M., et al., *Phase II multicentre study of temozolomide in combination with interferon alpha-2b in metastatic malignant melanoma.* Melanoma Res., 2006. **16**(4): p. 365-70.
26. Gogas, H., et al., *Temozolomide in combination with celecoxib in patients with advanced melanoma. A phase II study of the Hellenic Cooperative Oncology Group.* Ann Oncol., 2006. **17**(12): p. 1835-41. Epub 2006 Sep 15.
27. Hyman, S.L., E. Arthur Shores, and K.N. North, *Learning disabilities in children with neurofibromatosis type 1: subtypes, cognitive profile, and attention-deficit-hyperactivity disorder.* Dev Med Child Neurol., 2006. **48**(12): p. 973-7.
28. Coude, F.X., et al., *Academic impairment is the most frequent complication of neurofibromatosis type-1 (NF1) in children.* Behav Genet., 2006. **36**(5): p. 660-4.
29. Hyman, S.L., A. Shores, and K.N. North, *The nature and frequency of cognitive deficits in children with neurofibromatosis type 1.* Neurology., 2005. **65**(7): p. 1037-44.
30. Sood, A., et al., *Pegylated interferon alfa 2b and oral ribavirin in patients with HCV-related cirrhosis.* Indian J Gastroenterol., 2006. **25**(6): p. 283-5.

13 APPENDICES

13.1 Appendix 1: Performance Status Scales

Karnofsky and Lansky performance scores are intended to be multiples of 10

Karnofsky	Score	Lansky
Normal, no complaints, no evidence of disease	100	Fully active, normal
Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
Cares for self, unable to carry on normal activity or do active work	70	Both greater restriction of and les time spent in play activity
Required occasional assistance but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
Disabled, requires special care and assistance.	40	Mostly in bed' participates in quiet activities
Severely disabled, hospitalization indicated. Death not imminent	30	In bed; needs assistance even for quiet play
Very sick, hospitalization indicated. Death not imminent	20	Often sleeping; play entirely limited to very passive activities
Moribund, fatal processes progressing rapidly	10	No play; does not get out of bed

13.2 Appendix 2: Dosage chart for celecoxib by weight.
Target dose = 10mg/kg/day

Weight	**AM dose**		**PM dose**	
10-15kg	100mg		-0-	
15-20kg	100mg		50mg	100mg on odd numbered calendar days only
20-25kg	100mg		100mg	
25-30kg	150mg	100mg on even # days and 200mg on odd # days	100mg	
30-35kg	200mg		100mg	
35-40kg	200mg		150mg	100mg on even # days and 200mg on odd # days
40-45kg	200mg		200mg	
45-55kg	300mg		200mg	
55-65kg	300mg		300mg	
65-75kg	400mg		300mg	
>75kg	400mg		400mg	

13.3 Appendix 3

The National Institute of Health (NIH) has created specific criteria for the diagnosis of NF-1. Two of these seven "Cardinal Clinical Features" are required for positive diagnosis (Huson, 1994).

- 6 or more café-au-lait macules over 5 mm in greatest diameter in pre-pubertal individuals and over 15 mm in greatest diameter in post-pubertal individuals
- 2 or more neurofibromas of any type or 1 plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Optic glioma
- 2 or more Lisch nodules (iris harmartomas)
- A distinctive osseous lesion such as sphenoid dysplasia or thinning of the long bone cortex with or without pseudarthrosis
- A first degree relative (parent, sibling, or offspring) with NF1 by the above criteria

13.4 Appendix 4: Psychological Evaluations

Evaluation will include a measure of cognitive abilities (WPPSI-III, WISC-IV or WAIS-IV), visual-spatial abilities (Beery VMI or D-KEFS Tower Test), attention and impulsivity (Conner's CPT), academic achievement (WJ-III), depression (Children's Depression Inventory or Beck Depression Inventory), executive function (BRIEF) and behavior (CBCL). Test selection is dependent upon age (see chart below).

2 years to 5 years	**6 years to 16 years**	**17 years and older**
WPPSI-III*	WISC-IV*	WAIS-IV*
Beery VMI	Beery VMI (up to 8 years, then D-KEFS Tower Test)	D-KEFS Tower Test
	Conner's CPT	Conner's CPT
BRIEF (age 5 and up)	BRIEF	
CBCL	CBCL	
	Children's Depression Inventory	Beck Depression Inventory
	WJ-III Letter-Word Identification, Calculation, Passage Comprehension & Applied Problems	WJ-III Letter-Word Identification, Calculation, Passage Comprehension & Applied Problems

* Completed annually

Description of Psychological Testing Battery

Wechsler Intelligence Scale for Children- Fourth Edition (WISC-IV)
The WISC-IV consists of nine subtests used to measure verbal intelligence, perceptual intelligence, full scale intelligence, working memory and processing speed.

Wechsler Preschool and Primary Scale of Intelligence- Third Edition (WPPSI-III)
The WPPSI-III consists of ten subtests used to measure verbal intelligence, perceptual intelligence, processing speed and general language.

Beery Test of Visual Motor Integration (Beery VMI)
The Beery VMI is a developmental sequence of geometric forms to be copied with paper and pencil. It is designed to assess the extent to which individuals can integrate their visual and motor abilities.

Delis-Kaplan Executive Function System (D-KEFS) Tower Test
A means of assessing several key executive functions, including spatial planning, rule learning, inhibition of impulsive and preservative responding and the ability to establish and maintain the instructional set, by constructing target towers.

Conner’s Continuous Performance Test (Conner’s CPT)
The Conner’s CPT is a vigilance or attention test that requires respondents to press a button when a specified letter is presented, or not press a button when a specified letter is presented. The CPT measures attentiveness and impulsivity.

Woodcock-Johnson Tests of Achievement- Third Edition (WJ-III)
The WJ-III standard achievement measures take an inventory of academic skills including reading and math.

Children’s Depression Inventory (CDI)
The Children’s depression inventory is a self-report measure that measures the extent and severity of depressive symptoms in children aged 7 to 17 years of age.

Beck Depression Inventory
The BDI is a 21-item self-report instrument measuring the severity of depression in adults and adolescents aged 13 years and older.