AWARD NUMBER: W81XWH-09-1-0120

TITLE: Pilot Study of Gleevec/Imatinib Mesylate (STI-571, NSC 716051) in Neurofibromatosis (NF1) Patients with Plexiform Neurofibromas

PRINCIPAL INVESTIGATOR: Kent A. Robertson, M.D., Ph.D.

CONTRACTING ORGANIZATION: Indiana University
Indianapolis, IN 46202-5130

REPORT DATE: March 2016

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
March 2016	Final	1 Apr 2009 - 31 Dec 2015

4. TITLE AND SUBTITLE
Pilot Study of Gleevec/Imatinib Mesylate (STI-571, NSC 716051) in Neurofibromatosis (NF1) Patients with Plexiform Neurofibromas

5a. CONTRACT NUMBER
W81XWH-09-1-0120

5b. GRANT NUMBER
W81XWH-09-1-0120

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kent A. Robertson, M.D., Ph.D.

E-Mail: krobert@iu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Indiana University
Office of Research Administration
980 Indiana Ave, Room 2232
Indianapolis, IN 46202

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This is a second Pilot Study to determine the efficacy of Gleevec in neurofibromatosis (NF1) patients with plexiform neurofibromas using new response assessment modalities with the secondary goals of assessing Gleevec toxicity, and characterizing markers of response. The rationale for this study arises from the response of human NF1 cells to Gleevec in vitro, as well as in patients including the experience in 37 NF1 patients treated with Gleevec in the initial pilot study. Twenty one patients were enrolled including 8 pediatric patients. Four were inevaluable and 17 were evaluable completing at least 6 months of treatment owing to the success of a novel dose escalation schedule for individual patients. Primary endpoint MRI's, and secondary endpoints safety monitoring, circulating cytokines and endothelial progenitor cell assays, and quality of life data were obtained and assays completed from evaluable patients. Proposed biopsies, pressure measures, and PET imaging were not indicated in the enrolled patients. Results from each of the secondary endpoint assays await final MRI measures which are in progress. Once MRI measures are completed, they will be correlated with each of the secondary measures as potential markers of response with anticipation of completion by the end of this calendar year.

15. SUBJECT TERMS
Neurofibromatosis Type 1, Plexiform neurofibroma, Gleevec, Imatinib, Volumetric MRI, endothelial progenitor cells.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	23	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

1. INTRODUCTION: This is a second Pilot Study to determine the efficacy of Gleevec® in neurofibromatosis (NF1) patients with plexiform neurofibromas using new response assessment modalities with the secondary goals of assessing Gleevec toxicity, and characterizing markers of response. The rationale for this study arises from the response of human and murine NF1 cells to Gleevec® in vitro, the response of a NF1 patient treated with Gleevec® for airway compression by a plexiform neurofibroma with a dramatic response not previously seen in NF1 therapy, and the experience in 37 NF1 patients treated with Gleevec® in the initial pilot study. Twenty one patients were enrolled including 8 pediatric patients. Four were inevaluable and 17 were evaluable completing at least 6 months of treatment owing to the success of a dose escalation schedule for individual patients. Primary endpoint MRI's, and secondary endpoints safety monitoring, circulating cytokines and endothelial progenitor cell assays, and quality of life data were obtained from evaluable patients. Proposed biopsies, pressure measurements, and PET imaging were not indicated in the enrolled patients. Results from each of the secondary endpoint assays have been completed and await final MRI measures which are in progress. Once MRI measures have been completed, they will be correlated with each of the secondary measures with anticipation of completion by the end of this calendar year.

2. KEYWORDS: Neurofibromatosis Type 1, Plexiform neurofibroma, Gleevec, Imatinib, Volumetric MRI, endothelial progenitor cells.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The **goal** of this Pilot Study is to trial and develop multiple techniques for determining the response of NF1 patients with plexiform neurofibromas to Gleevec® therapy for use in subsequent clinical trials. The **impact** of this proposal is having a better way to measure/quantify the response of plexiform neurofibromas in NF1 patients to treatment.

Funded 10/17/08

Completed 8/10/16

Task 1. Complete writing and submission of clinical trial for approval to the Scientific Review Committee (SRC), Radiation Safety Committee (RSC), Institutional Review Board (IRB), Novartis, and the DOD Human Research Protections Office (HRPO) (Months 1-9):

a. Finalize Protocol submit and receive approval from Indiana School of Medicine SRC, RSC, IRB, and Novartis approval (Months 1-6).
b. Submission and approval of locally approved protocol to HRPO (Months 6-9).
c. Identify prospective NF1 patients for enrollment upon approval (Months 1-9).

Task 2. Consent and enroll patients on study; initiate therapy with Gleevec with proposed monitoring and evaluation of response (Months 10-36).

a. Enroll and initiate treatment of eligible patients per approved protocol (Months 10-24).
b. Submit Annual reports. (Months 10-24).
c. Anticipated that all 20 patients will be enrolled by month 24 with the final 12 months required to complete therapy and the assays/analyses to determine response (Months 24-36).
d. Complete cytokine assays, volumetric analyses, QOL tool assessment-analysis, flow analysis compilation, MRI analysis, analysis of PET imaging data, histology review, and bioactivity assays (Months 30-36).
e. Prepare final report and data for publication (Months 30-36).

The protocol was submitted to the Indiana University Cancer Center Scientific Review board on July 31, 2009 with approval on Oct 7, 2009. The protocol was also submitted to the Indiana University Institutional Review Board on July 31, 2009 and approved 10/29/2009. These documents were submitted to the DOD on 11/2009. We received the DOD provisions on 1/19/2010 and returned the response to the provisions on 2/18/2010. The protocol was amended to include the provisions from the DOD and Novartis and submitted to our IRB 3/16/2010. We received IRB approval of Amendment #1 on 3/29/2010. We received final DOD approval 6/4/2010. We enrolled our first subject on 6/30/2010. Because of slow enrollment, we applied and obtained an amendment to enroll pediatric patients. We have enrolled a total of 21 patients with amending the study to include pediatric patients. We received a no-cost extension to complete enrollment of the pediatric cohort. The last patient was enrolled on 02 June 2014 with last patient visit was on 22-JUN-2015. High resolution flow cytometry data for endothelial progenitor cells was collected real time. Cytokine analysis of all samples were run together and began in May 2015 and completed assays in May 2016 with change from ELISA format to EMD Millipore Multiplex. Quality of Life (QOL) data analysis was completed April 2016. The Indiana IRB approved a renewal on 21-Jul-2016, closed to enrollment, for data analysis. MRI analysis is ongoing and upon completion will be used to correlate response with endothelial cell progenitor populations, cytokines, and QOL as potential measures of response.

What was accomplished under these goals?

1. Enrollment. Approval and initiation of enrollment was accomplished in a timely manner. However, over time enrollment was slow. To approach this problem, we submitted an amendment to allow treatment of children resulting in our last 8 patients enrolled being children.

A. Enrollment Timing:

ID#	MRN	First Name	Last Name	enroll date	month 7	month 13
NF1DOD-01		Jo		6/30/2010	12/13/2010	8/8/2011
NF1DOD-02		La		9/20/2010	3/7/2011	11/7/2011
NF1DOD-03		Er		9/24/2010	3/11/2011	8/12/2011
NF1DOD-04		Ti		10/11/2010	5/16/2011	11/21/2011
NF1DOD-05		Ma		1/18/2011		
NF1DOD-06		Br		1/24/2011	7/18/2011	1/9/2012
NF1DOD-07		Te		2/23/2011	11/14/2011	**withdrew**
NF1DOD-08		Ro		3/4/2011	11/10/2011	6/15/2012
NF1DOD-09		Ke		4/18/2011	12/5/2011	
NF1DOD-10		Ma		5/3/2011	11/3/2011	
NF1DOD-11		Je		7/8/2011		
NF1DOD-12		Va		6/20/2011	12/2/2011	6/19/2012
NF1DOD-13		Je		7/1/2011	12/16/2011	6/4/2012
NF1DOD-14		Sa		3/26/2012	9/24/2012	4/22/2013
NF1DOD-15		Sc		4/18/2012	10/22/2012	
NF1DOD-16		Br		6/19/2012	12/17/2012	6/28/2013
NF1DOD-17		Ja		1/28/2013	6/24/2013	2/3/2014
NF1DOD-18		Ji		2/26/2013	8/26/2013	lost to follow up
NF1DOD-19		Ar		3/11/2012	9/11/2013	3/10/2014
NF1DOD-20		Mi		9/26/2013	3/21/2013	inevaluable
NF1DOD-21		Ol		6/3/2014	12/8/2014	6/22/2015

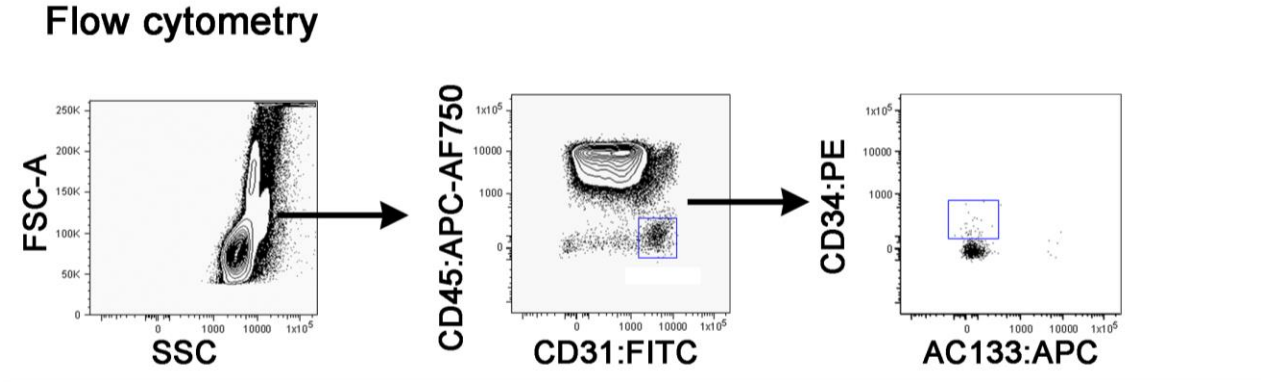
2. Real time high resolution flow cytometric evaluation of circulating endothelial progenitors.

Flow for Endothelial Progenitor Cells

EPCs are being studied as surrogate markers for multiple vascular pathologies in adults. However, data interpretation has been difficult because of inconsistent definitions and methodologies used to enumerate these rare circulating cells. Flow cytometry has been used to

identify and enumerate circulating EPC subpopulations as outlined below. Specifically, Duda et. al. standardized, in a recent *Nature Protocols* paper, a flow cytometry-based method for enumerating specific EPC populations in blood that serve as biomarkers for vascular disease risk and response to anti-angiogenic therapies in human cancers. MNCs are stained with antibodies directed against endothelial and progenitor cell-specific antigens. Viable MNCs are analyzed for CD34+CD133+CD45dimCD31+, circulating progenitor cells (CPCs) and CD34+CD45-CD31+CD133-, circulating endothelial cells (CECs), which correlate with disease progression in various pathologies. However, no previous studies have been conducted in patients with NF1. Examination of these cell types is important, especially in patients with plexiform nuerofibromas, as these cells types may potentiate angiogenesis.

The Angiogenesis and Endothelial Progenitor Cell Core at Indiana University, directed by Dr. Ingram, developed a standardized 5-color flow cytometry method based on recent studies by Duda et. al. The first fluorescent channel is a "dump channel", which is used to exclude non-viable cells, red blood cells, and platelets from analysis. This is a key step since contamination with these cell populations can significantly alter the accuracy of the data generated, especially when rare populations are analyzed. The remaining 4 antigens examined are CD34 and CD133 (stem and progenitor cell antigens), CD45 (hematopoietic cell antigen), and CD31 (endothelial cell antigen). Gating strategies are illustrated below.



Flow Cytometric Method: A 5-color flow cytometry assay will be conducted similar to preliminary studies. MNCs will be stained with antibodies against cell surface antigens, CD34, CD133, CD31, and CD45, as well as a viability marker, CD41a, and glycophorin A for the exclusion of dead cells, platelets, and red blood cells, respectively. Fluorescence minus one (FMO) controls will be prepared as negative gating controls. The frequency of phenotypically defined cell populations will be analyzed using an LSR II flow cytometer and FlowJo software. Data has been obtained for all 21 patients. Multiple time points were obtained in all but 3 patients. Time points were assayed as available at baseline, 2-3 weeks, and 6 months of Gleevec therapy. Of some interest, patients developing edema had a large increase in their circulating progenitor cell CPC ratio in a short period. Additionally, one patient with an airway tumor had a significant increase in their CPC ratio over time after a stable ratio from baseline

and 2 weeks. Data will be correlated with MRI response data to see if trends in CPC ratios correlate with or are predictive of response (better or worse).

3. Bio-Plex Circulating Cytokine Analysis.

Funds unused from tumor biopsy immunohistochemistry were used to expand the number of circulating cytokines assayed and change from the more expensive ELISA based assay to the more economical Millipore Bio-Plex system. Part of the incentive to expand this panel came from data in our initial pilot study with Gleevec in NF1 plexiforms using a 15 cytokine ELISA panel which demonstrated that a greater than two-fold increase in certain circulating cytokines reflected tumors that progress. This study's cytokine assay employed the MilliPlex map Human Cytokine/Chemokine Magnetic Bead Panel (cat# HCYTMAG-60K-PX38). Assayed cytokines included sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF , GRO, IFN-α2 , IFN-γ, IL-1α , IL-1β, IL-1ra , IL-2 , IL-3 , IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 , IL-10 , IL-12 (p40) , IL-12 (p70) , IL-13, IL-15 , IL-17 , IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1$^{\alpha}$, MIP-1β, TGF-α, TNF-α, TNF-β, VEGF, PDGF AA, PDGF AB/BB, and RANTES. Soluble cytokine receptors were assayed using MILLIPLEX MAP Human Soluble Cytokine Receptor Panel (cat# HSCRMAG-32K) and included: sCD30, sgp130, sIL-1RI, sIL-1RII, sIL-2Rα, sIL-4R, sIL-6R, sRAGE, sTNFRI, sTNFRII, sVEGF-R1, sVEGF-R2, sVEGF-R3. Separately were run TGF β 1, TGF β 2, and TGF β 3 using the MILLIPLEX MAP TGFß Magnetic Bead 3 Plex Kit (cat# TGFBMAG-64K-03). And finally, SCF, an important target in growing plexiform tumors, was assayed using the MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel II (cat# HCYP2MAG-62K).

Samples were aliquoted for each kit and had 2 freeze/thaw cycles to keep the freeze/thaw variable consistent throughout all kits and plates. Samples were aliquoted due to some samples only have 1-2 vials of plasma. Therefore 1 vial was thawed and the plasma was aliquoted into new, sterile, tubes for each kit. Each new aliquot was then frozen until ready for use. All samples have been assayed. Data will be correlated with MRI response data to see if trends in circulating cytokines, cytokine receptors, TGF, and SCF correlate with or are predictive of response to therapeutic intervention.

4. Quality of Life (QOL) Analysis.

Adult QOL was measured using the SF 36. This instrument is a modular instrument for measuring health-related QOL in adults. The SF 36 Core Scales are multidimensional self-report scales developed as a generic core measure of QOL. The SF 36 Core Scales measure physical functioning, emotional functioning, social functioning. Validity of the SF 36 Core Scales was established by known group comparisons and correlations with other measures of disease burden. Reliability was established by internal consistency reliability. Alpha coefficients for self-report were greater than 0.90. Item response distributions were across the full-scale range, with no floor effects and minimal ceiling effects.

Short Form 36 version 2 (SF-36v2): N=12 at baseline

Table 1: Mean Domain Scores of Study sample compared with US Normative population

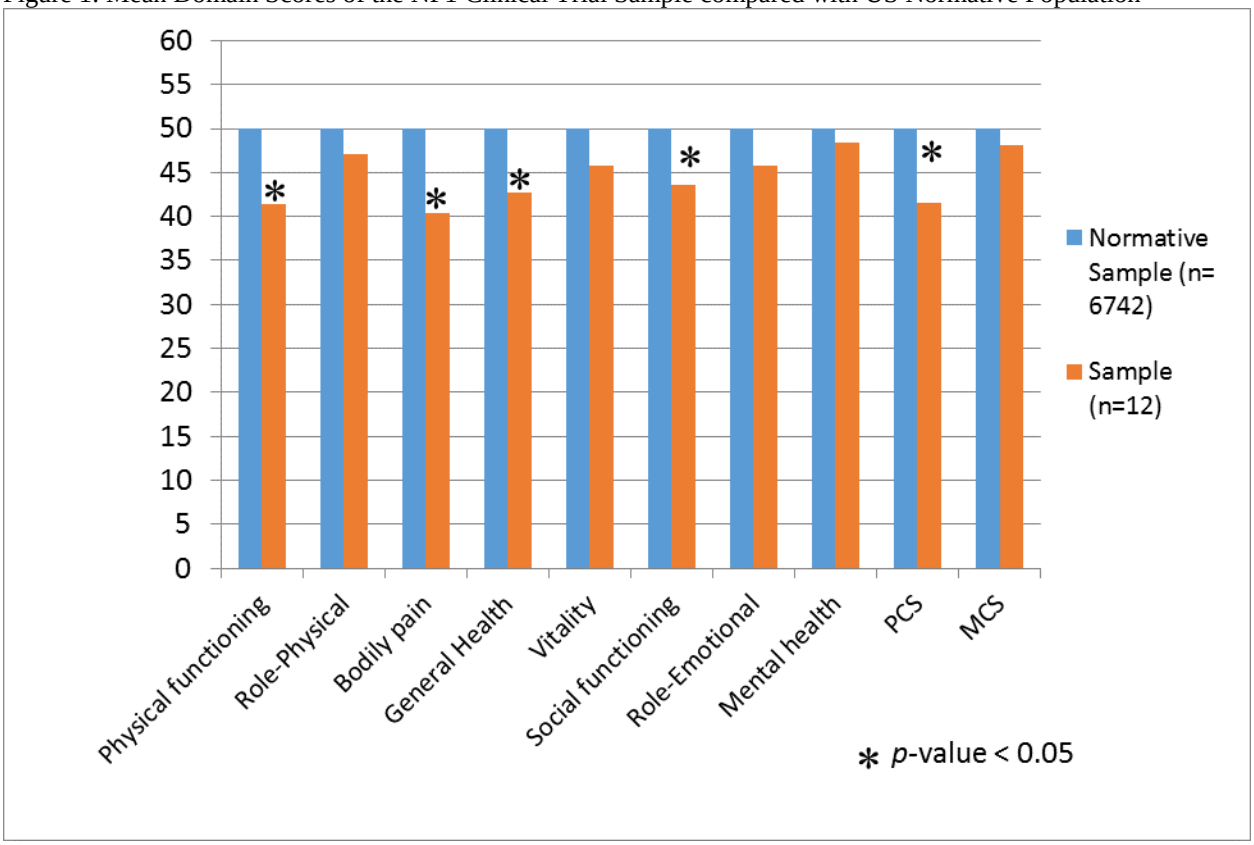
Domain	Study sample (n=12)	Normative population (n=6742)	*P*-value
Physical Functioning	41.43 ± 13.08	50 ± 10	.003*
Role-Physical	47.09 ± 11.58	50 ± 10	.314
Bodily Pain	40.38 ± 13.43	50 ± 10	.001*
General Health	42.7 ± 11.76	50 ± 10	.012*
Vitality	45.85 ± 14.69	50 ± 10	.151
Social Functioning	43.64 ± 15.14	50 ± 10	.028*
Role-Emotional	45.84 ± 11.54	50 ± 10	.150
Mental Health	48.37 ± 11.86	50 ± 10	.573
Physical Component Summary Score	41.53 ± 14.04	50 ± 9.95	.003*
Mental Component Summary Score	48.09 ± 13.97	50 ± 10.03	.510

**p*-value <.05 & statistically significant

Table 2: Mean scale scores of the study sample at the baseline and at 6 months after the beginning of clinical trial (n =6)

Domain	Baseline scores	Follow up scores	*p*-value
Physical functioning	45.5 ± 13.6	47.2 ± 10.7	.61
Role-Physical	49.1 ± 11	43.8 ± 11.4	.12
Bodily pain	41.4 ± 16.4	41.4 ± 14.2	1.00
General Health	45.1 ± 15.3	49.7 ± 11.6	.53
Vitality	43.8 ± 19.5	39.8 ± 13.5	.31
Social functioning	43.2 ± 15.3	43.2 ± 15.7	1.00
Role-Emotional	43.6 ± 15	37.8 ± 15.9	.12
Mental health	46.7 ± 13.5	43.9 ± 12.5	.32
PCS	45.6 ± 13.9	50.4 ± 7.1	.45
MCS	44.4 ± 16.7	41.9 ± 17.3	.74

Figure 1: Mean Domain Scores of the NF1 Clinical Trial Sample compared with US Normative Population



The Pediatric Quality of Life Inventory (PedsQL) tool is designed to measure health-related quality of life in children and adolescents aged 2–18 years. In our study, the Pediatric QOL evaluation was performed using the PedsQL tool in 5 pediatric patients:

Gleevec Trial PedsQL Generic Core Scales

Patient ID	Baseline	6 month F/U	12 month F/U
NF1DOD-14	Y	Y	Y
Gleevec-16		Y	Y
Gleevec-17	Y	Y	Y
Gleevec-19	Y	Y	Y

Gleevec-21	Y	Y	Y

PedsQL – four scales

1. Health and activities (Physical functioning)
2. Feelings (Emotional functioning)
3. Get along with others (Social functioning)
4. School functioning

Scale Scores

Scale(n=4)	**Baseline Scores**
Physical Functioning	82.03 ± 10.32
Emotional Functioning	73.75 ± 18.87
Social Functioning	85 ± 5.77
School Functioning	54.17 ± 26.02
Total Scale Score	77.32 ± 7.82

Scale (n=5)	**6mon F/U**
Physical Functioning	90.63 ± 10.36
Emotional Functioning	91 ± 12.45
Social Functioning	94 ± 8.22
School Functioning	90 ± 12.25
Total Scale Score	91.45 ± 8.4

Scale (n=5)	**12mon F/U**
Physical Functioning	80 ± 16.02
Emotional Functioning	92 ± 13.04
Social Functioning	82 ± 13.51
School Functioning	73.33 ± 28.29
Total Scale Score	82.56 ± 15.29

Paired t-test for baseline and 6-month follow up scores on PedsQL

Scale (n=4)	**Baseline**	**6mon F/U**	**p-value**	**Effect Size**
Physical Functioning	82.03 ± 10.32	91.41 ± 11.8	0.05	0.8
Emotional Functioning	73.75 ± 18.87	88.75 ± 13.15	0.13	1.1
Social Functioning	85 ± 5.77	92.5 ± 8.66	**0.01***	0.9
School Functioning	54.17 ± 26.02	86.67 ± 12.58	0.11	2.6
Total Scale Score	77.32 ± 7.82	90.4 ± 9.31	**0.02***	1.4

*Social Functioning and Total scale scores showed statistically significant improvement from baseline to 6 mon follow up.

Paired t-test for baseline and 12 month follow up scores on PedsQL

Scale (n=4)	Baseline	12 mon F/U	p-value
Physical Functioning	82.03 ± 10.32	76.57 ± 16.24	0.64
Emotional Functioning	73.75 ± 18.87	90 ± 14.14	0.23
Social Functioning	85 ± 5.77	77.5 ± 10.41	0.3
School Functioning	54.17 ± 26.02	64.44 ± 26.94	0.11
Total Scale Score	77.32 ± 7.82	78.75 ± 14.65	0.85

ANOVA comparing three baseline, 6 month and 12 month follow up scores on PedsQL

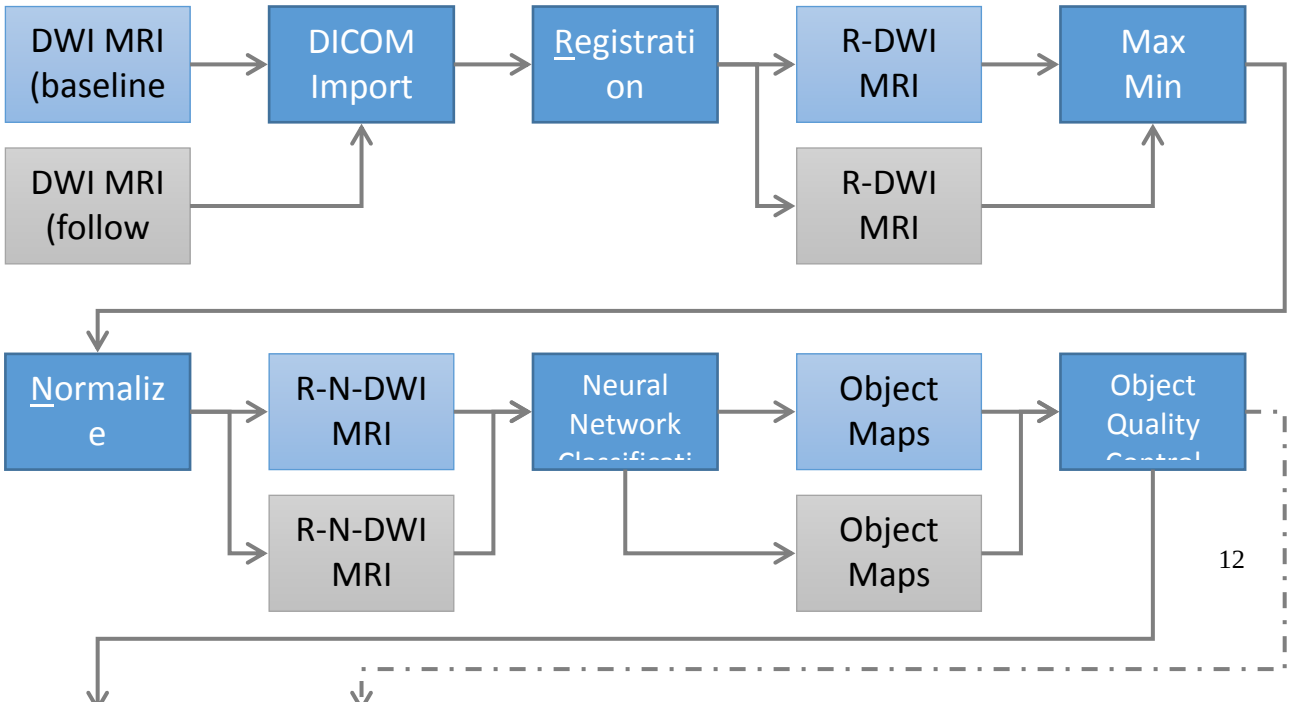
Scale (n=4)	Baseline	6mon F/U	12 mon F/U	p-value
Physical Functioning	82.03 ± 10.32	91.41 ± 11.8	76.57 ± 16.24	0.31
Emotional Functioning	73.75 ± 18.87	88.75 ± 13.15	90 ± 14.14	0.31
Social Functioning	85 ± 5.77	92.5 ± 8.66	77.5 ± 10.41	0.09
School Functioning	54.17 ± 26.02	86.67 ± 12.58	64.44 ± 26.94	0.28
Total Scale Score	77.32 ± 7.82	90.4 ± 9.31	78.75 ± 14.65	0.24

QOL data will be correlated with MRI response data to see if trends correlate with response to therapeutic intervention.

5. Magnetic Resonance Imaging Analysis.

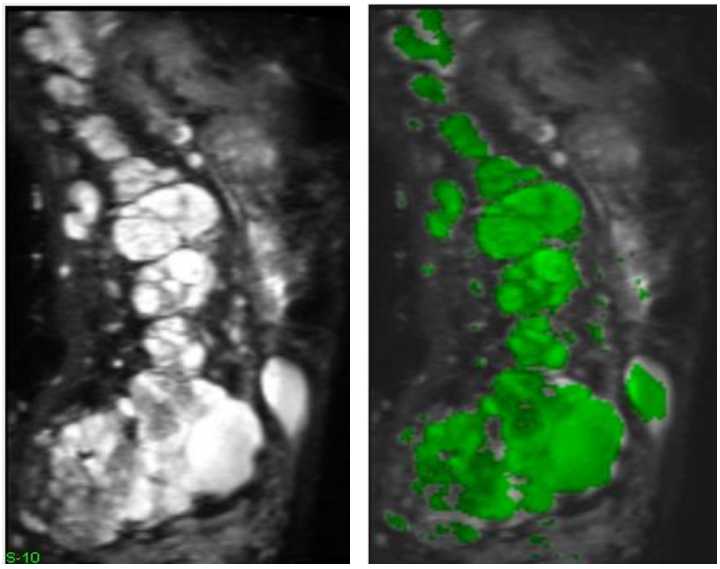
Tumor response criteria that are used for cancers are based on one-dimensional (1-D) and two-dimensional (2-D) tumor measurements. These methods have limited value in the assessment of treatment outcome for plexiform neurofibromas, which are frequently large, have a complex (non-spherical) shape, and have a slow growth pattern.

In order to reproducibly quantify the size of these complex lesions and detect small changes in the size over time, our imaging group including Drs. Hutchins and Territo and Mr. Persohn have established tumor segmentation technique that takes each imaging through a sequence of analyses to yield volumetric information and diffusion modeling. Where this becomes valuable

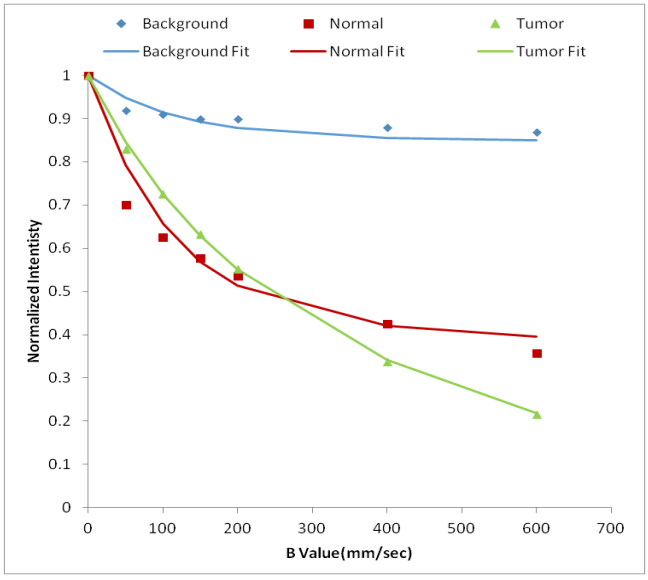


-
-
-

is in characterizing the fluid dynamics and potential for drug perfusion of plexiform tumors that have developed a more extensive collagenous or vascular microenvironment which can impact drug delivery. Tumor tissue is often difficult to distinguish from normal structures, but applying a neural network multispectral classification plot for segmentation can allow differentiation between normal and tumor tissue by differences in their very different slopes:



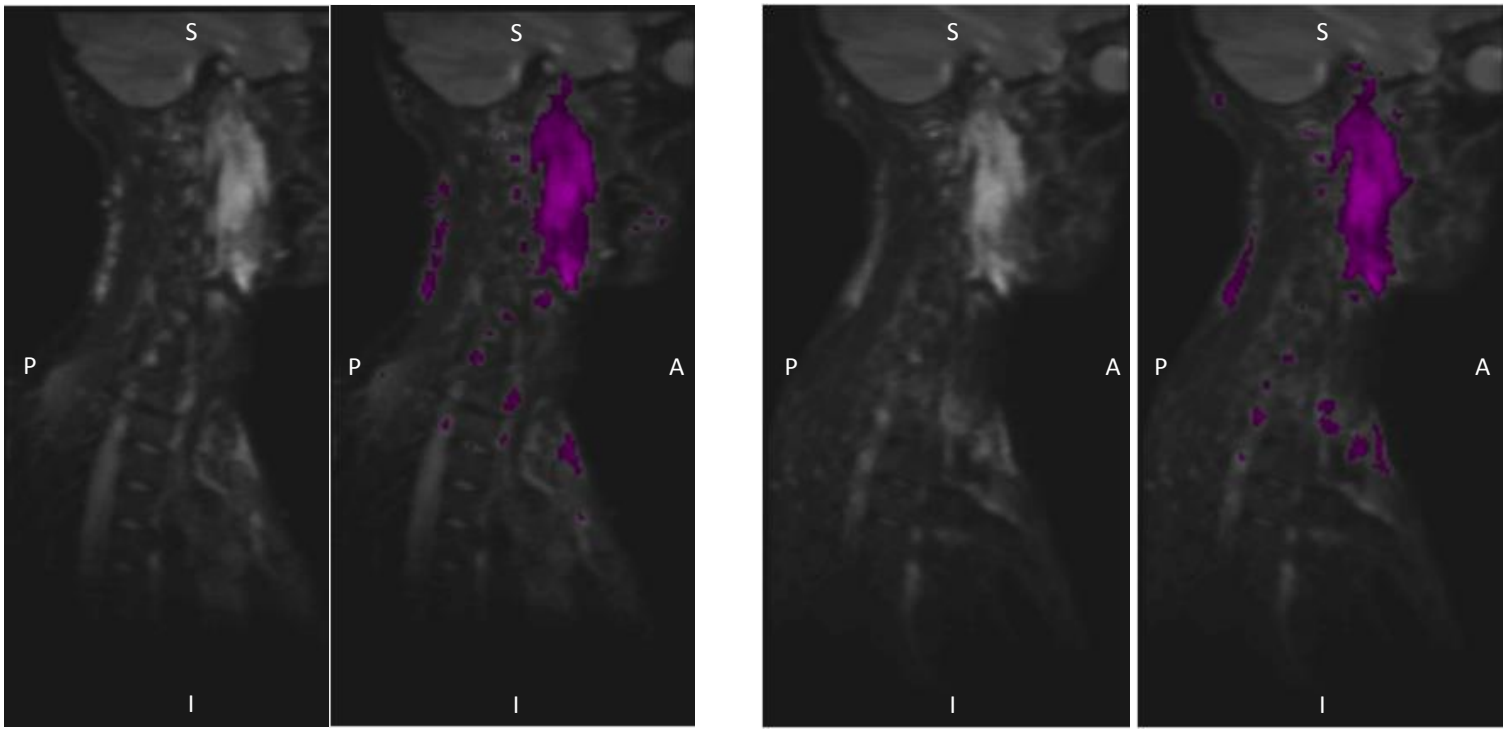
DWI (B0) Tumor



In the next 2 sets of images, we pulled together representative cases for the NF1 tumors pre and post Gleevec treatment. The left of each of these images is the B0 DWI MRI to show anatomy and the right is the same image with the tumors highlighted in fuchsia/pink. These tumors although not always smaller do seem to have difference appearance (less textured) in the post treatment groups. At the current time, we can't say much about the volumes for these as we have not finished the analysis and statistics, but this will be forthcoming soon. In addition, we plan to use all 7 B-value DWI images and compute the IVIM diffusion model for these same subjects. This will tell us if the molecular composition of the tumor has changed with respect to

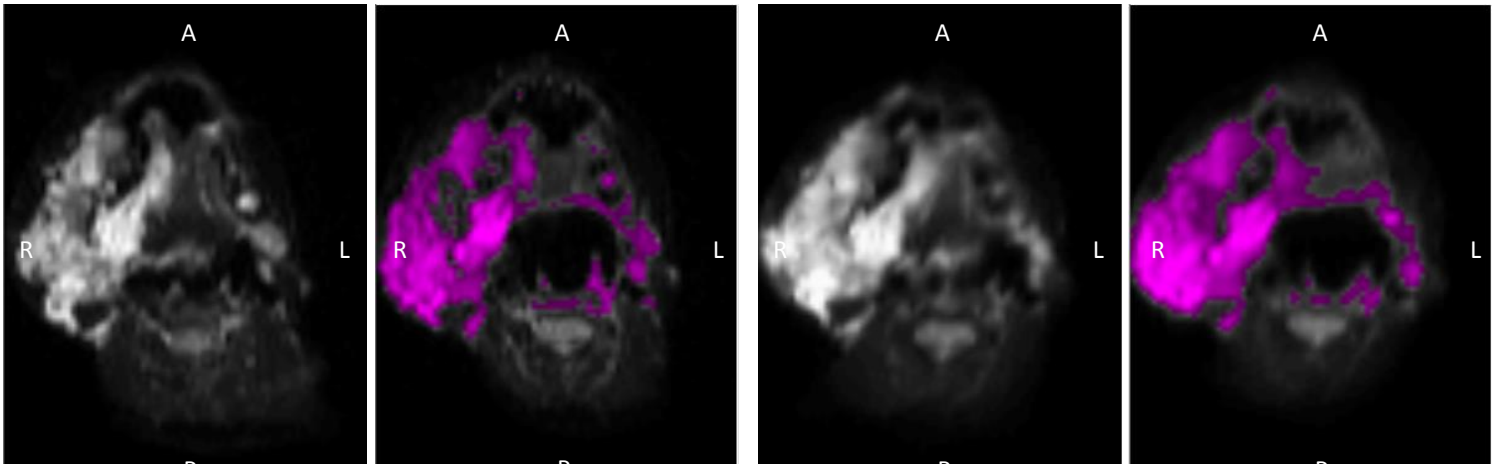
diffusion and tissue perfusion. Our hope is that this measure may provide valuable insights into the changes that Gleevec can have on NF1 tumors which may be independent of tumor volumes. Volumetric data generated will be correlated with cytokine, EPC, and QOL data to determine impact of tumor response on other measures of response.

Neurofibromatosis Pre and Post Gleevec (NF13)



Pre Treatment (Anatomy) | Pre Treatment (Tumor) | Post Treatment (Anatomy) | Post Treatment (Tumor)

Neurofibromatosis Pre and Post Gleevec (NF17)



What opportunities for training and professional development has the project provided? Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals? This is the final report, however MRI analyses are ongoing and projected to be completed by the end of the calendar year along with correlation with circulating cytokines and endothelial progenitor cells, and Quality of Life (QOL) to determine utility of these parameters as measures of tumor response. A formal follow-up report along with progress on a manuscript will be forwarded to the DOD at that time.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Response to therapy has been assessed using multiple techniques that have been developed in molecular and cellular characterization of these tumors or techniques developed to more carefully measure tumor size. These endpoints/techniques/**specific aims** before and after Gleevec include 1) conventional MRI measurements, 2)volumetric MRI measurements, 3) circulating endothelial progenitor cells by flow cytometry, and 4) circulating cytokine levels including SCF, TGF-β, PDGF, and midkine. Additionally, because there are few published studies evaluating clinical symptoms/quality of life in NF1 patients, we have utilized this group of patients to characterize health related quality of life measures to evaluate symptoms and the functional impact of their disease on their lives. Ultimately this kind of evaluation would be used for determining the impact of therapies on plexiform neurofibromas as it influences patient's lives. The **goal** of this Pilot Study has been to trial and develop multiple techniques for

determining the response of NF1 patients with plexiform neurofibromas to Gleevec therapy for use in subsequent clinical trials. The **impact** of this proposal is having a better way to measure/quantify the response of plexiform neurofibromas in NF1 patients to therapies to identify which treatments will work to benefit patients to the greatest extent. These methods of measurement provide a way to ask with each clinical trial, how can we make NF1 plexiform neurofibromas respond better.

What was the impact on other disciplines? Potential application of these measures in other tumor systems.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology?

Plexiform tumors are among the most frustrating tumors to treat because they respond so poorly to conventional chemotherapy and produce chronic pain and slow progressive impingement on normal organs which in some cases are life threatening. By improving therapy for these awful tumors, we can move toward an improved the quality of life in patients with neurofibromatosis that occur in one in 3500 people.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change. The plexiform tumors encountered in this study did not present as indications for biopsy which precluded the immunohistochemical tissue studies. These resources were utilized for an expanded cytokine array as an economical and efficient method to probe the activity and response of plexiform tumors to Gleevec.

Actual or anticipated problems or delays and actions or plans to resolve them. Because of slow accrual, an amendment was submitted and approved by the DOD and IRB to allow enrollment of pediatric patients which significantly improved enrollment allowing completion of the study.

Changes that had a significant impact on expenditures . As above.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. Nothing to report.

Significant changes in use or care of human subjects. Nothing to report.

Significant changes in use or care of vertebrate animals. Nothing to report.

Significant changes in use of biohazards and/or select agents. Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications. Nothing to report.

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers, and presentations. Nothing to report.

Website(s) or other Internet site(s). Nothing to report.

Technologies or techniques. Analysis of the MRI scans for this trial has utilized the application of a novel MRI volumetric technique to assess size and density of plexiform tumors. A separate publication for dissemination will be generated with this technique and appropriate citing application/use in the CDMRP-DOD sponsored trial.

Inventions, patent applications, and/or licenses. Nothing to report.

Other Products. Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Kent Robertson, M.D.

Role: Principle Investigator

Person-months-worked per year: 3

Contribution to Project: Oversight of Entire Project

Name: Mervyn Cohen, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.36

Contribution to Project: X-ray reading

Name: James Fletcher, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.24

Contribution to Project: PET Imaging Reading

Name: Chang HO, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.48

Contribution to Project: MRI Interpretation

Name: David Ingram, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.6

Contribution to Project: Oversight of Endothelial Progenitor Flow Cytometry

Name: Chi-Schin Shih, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.6

Contribution to Project: Clinical Care of patients

Name: Nancy Swigonski, M.D.

Role: Co- Investigator

Person-months-worked per year: 1.3

Contribution to Project: Quality of Life Assessments

Name: Jeff Travers, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.24

Contribution to Project: Biopsy of skin based tumors

Name: Feng-Chun Yang, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.6

Contribution to Project: Cytokine Assays

Name: Menggang Yu, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.6

Contribution to Project: Bio-statistical Analysis

Name: Francis Marshelleck, M.D.

Role: Co- Investigator

Person-months-worked per year: 0.6

Contribution to Project: Biopsy of non-skin based plexiform tumors

Name: Yan Li, B.S.

Role: Research Tech

Person-months-worked per year: 3

Contribution to Project: Cytokine assays

Name: Julie Mund, M.S.

Role: Research Manager

Person-months-worked per year: 3

Contribution to Project: Endothelial Progenitor Flow Lab and assays

Name: Cindy Elkins, RN

Role: Research Nurse

Person-months-worked per year: 2.4

Contribution to Project: Enrollment of Patients

Name: Marcie Sherman, M.S., B.S.

Role: Clinical Research Coordinator

Person-months-worked per year: 2.4

Contribution to Project: Enrollment and data management

Name: Paul Territo, Ph.D.

Role: Co- Investigator

Person-months-worked per year: 1.2

Contribution to Project: MRI Image analysis

Name: Gary Hutchins, Ph.D.

Role: Co- Investigator

Person-months-worked per year: 1.2

Contribution to Project: Oversight of MRI Imaging Group

Name: Scott Persohn, B.S.

Role: Radiology MRI Tech

Person-months-worked per year: 1.2

Contribution to Project: MRI Image analysis

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to report.

What other organizations were involved as partners? Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Nothing to report.

QUAD CHARTS: Nothing to report.

9. APPENDICES:

Manuscript on which Dr. Robertson was a co-author putting forth recommendations for MRI response endpoints in neurofibromatosis clinical trials.

Recommendations for imaging tumor response in neurofibromatosis clinical trials

Eva Dombi, MD
Simone L. Ardern-Holmes, MD
Dusica Babovic-Vuksanovic, MD
Fred G. Barker, MD
Steve Connor, MD
D. Gareth Evans, MD
Michael J. Fisher, MD
Stephane Goutagny, MD, PhD
Gordon J. Harris, PhD
Diego Jaramillo, MD
Matthias A. Karajannis, MD
Bruce R. Korf, MD, PhD
Victor Mautner, MD
Scott R. Plotkin, MD, PhD
Tina Y. Poussaint, MD
Kent Robertson, MD, PhD
Chie-Schin Shih, MD
Brigitte C. Widemann, MD
For the REiNS International

ABSTRACT

Objective: Neurofibromatosis (NF)-related benign tumors such as plexiform neurofibromas (PN) and vestibular schwannomas (VS) can cause substantial morbidity. Clinical trials directed at these tumors have become available. Due to differences in disease manifestations and the natural history of NF-related tumors, response criteria used for solid cancers (1-dimensional/RECIST [Response Evaluation Criteria in Solid Tumors] and bidimensional/World Health Organization) have limited applicability. No standardized response criteria for benign NF tumors exist. The goal of the Tumor Measurement Working Group of the REiNS (Response Evaluation in Neurofibromatosis and Schwannomatosis) committee is to propose consensus guidelines for the evaluation of imaging response in clinical trials for NF tumors.

Methods: Currently used imaging endpoints, designs of NF clinical trials, and knowledge of the natural history of NF-related tumors, in particular PN and VS, were reviewed. Consensus recommendations for response evaluation for future studies were developed based on this review and the expertise of group members.

Results: MRI with volumetric analysis is recommended to sensitively and reproducibly evaluate changes in tumor size in clinical trials. Volumetric analysis requires adherence to specific imaging recommendations. A 20% volume change was chosen to indicate a decrease or increase in tumor size. Use of these criteria in future trials will enable meaningful comparison of results across studies.

Conclusions: The proposed imaging response evaluation guidelines, along with validated clinical outcome measures, will maximize the ability to identify potentially active agents for patients with NF and benign tumors. *Neurology*® 2013;81 (Suppl 1):S33–S40

GLOSSARY

CR = complete response; **NF** = neurofibromatosis; **PD** = progressive disease; **PN** = plexiform neurofibroma; **PR** = partial response; **RECIST** = Response Evaluation Critera in Solid Tumors; **REiNS** = Response Evaluation in Neurofibromatosis and Schwannomatosis; **SD** = stable disease; **STIR** = short TI inversion recovery; **TTP** = time to progression; **VS** = vestibular schwannoma; **WHO** = World Health Organization.