Neurofibromatosis (NF) Consortium

NF PROTOCOL 108

A Phase II Study of Binimetinib in Children and Adults with NF1 associated Plexiform Neurofibromas (PNOC010)

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ABSTRACT/SCHEMA

Background

- Patients with Neurofibromatosis 1 (NF1) have an increased risk of developing tumors of the peripheral nervous system, including plexiform neurofibromas (PN), which are benign nerve sheath tumors that are among the most debilitating complications of NF1. There are no standard treatment options for PN other than surgery, which is often difficult due to the extensive growth and invasion of surrounding tissues.
- The Mitogen-activated Protein Kinase (MAPK) pathway is important for plexiform neurofibroma growth.
- Phosphorylated Extracellular signal-regulated protein kinase (p-ERK) is detected in human plexiform neurofibromas at higher levels than normal human nerve.
- The *Dhh-Cre;Nf1fl/fl* mouse model develops plexiform neurofibromas, and these tumors histologically mimic those of human plexiform neurofibromas [1].
- Elevated phosphorylated (p)-ERK1,2 was detected in *Nf1fl/fl;Dhh-cre* mouse neurofibromas relative to *Nf1fl/fl;Dhh-cre* or wild type mouse nerve.
- Binimetinib is an ATP-uncompetitive inhibitor of MEK1/2 currently in clinical cancer trials that blocks MAPK signaling.

Primary Objective

- To determine the proportion of children and adults with NF1 associated plexiform neurofibromas who will have an objective response to binimetinib defined as a 20% or greater decrease in tumor volume reduction by 12 courses.

Secondary Objectives

- To evaluate the feasibility and toxicity of protracted binimetinib administration in this patient population.

Exploratory Aims

- To evaluate the effect of binimetinib on quality of life and pain.
- To validate the NRS-11, which has been adapted to assess pain intensity in individuals with NF1 and plexiform neurofibromas, for use in this patient population
- To characterize the effect of binimetinib on plasma cytokines and growth factors
- To determine whether subjects who respond will maintain that response for 1 year once they come off therapy.
- To estimate the objective response rate of up to 2 non-target plexiform neurofibromas to binimetinib by MRI.
- To evaluate the effect of binimetinib on functional outcomes depending on PN location, such as vision, motor, disfigurement, bowel/bladder
- To obtain blood and tumor tissue to be stored for future PN biological studies
- To compare the volumetric assessment of plexiform neurofibroma response to therapy performed by two independent analysts using different volumetric analysis methods.
- To evaluate the likelihood of response and prolonged disease stabilization rate to binimetinib in participants with recurrent, progressive PN who previously responded to binimetinib.

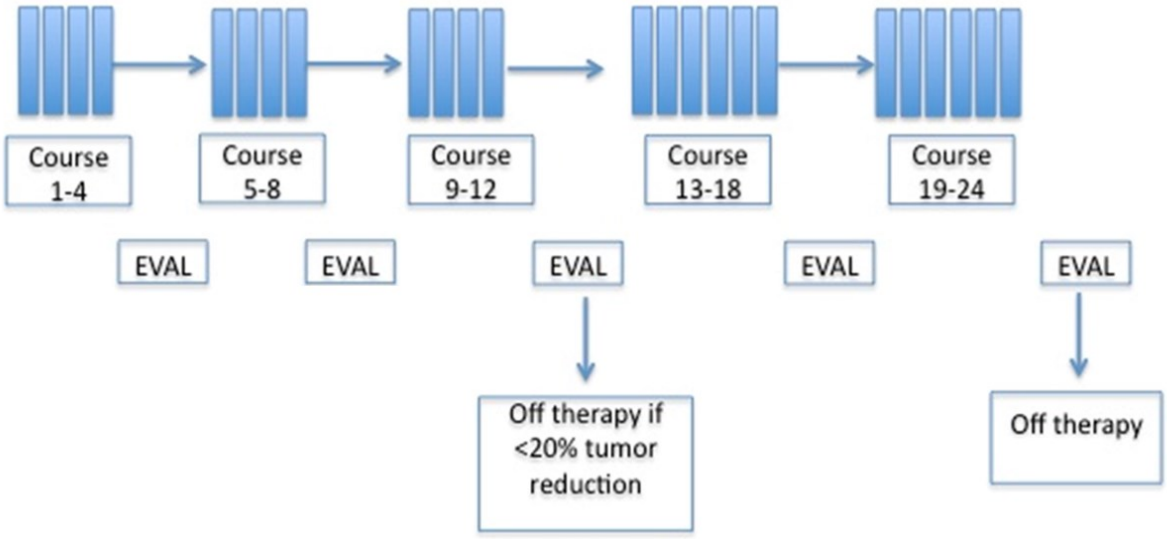
Eligibility

- Patients ≥1 year of age with NF1 and PN(s) that are progressive by serial imaging OR causing significant morbidity, and that can be analyzed by volumetric MRI.

Design

- Binimetinib starting dose was initially 45 mg per dose (the adult RP2D) orally twice daily in the adult stratum (Stratum A) but was reduced (since protocol version 2.0) to 30 mg per dose twice daily. The starting dose in the pediatric stratum (Stratum B) is 32 mg/m^2 per dose (maximum 45mg per dose), which has been established as the pediatric RP2D by NCT02285439 (PI: Robison). Dosing will be continuous, with 28 days defined as a course, and will continue for a total of 24 courses or until one of the Off Treatment or Off Study criteria are met.
- Subjects will undergo volumetric assays of their target PN using MRI after every 4 courses for the first year and then every 6 courses. Review of the volumetric analysis will occur centrally at the NCI under the guidance of Dr. Dombi.
- Treatment beyond course 12 will only be given to those subjects who achieve a response (≥ 20% tumor shrinkage by volumetric analysis) by the end of course 12, and will be continued for a maximum of 24 total courses. Subjects will be removed for significant toxicity, treatment delay ≥ 28 days, or objective progression (≥ 20% tumor growth by volumetric analysis) at any time.

EXPERIMENTAL DESIGN SCHEMA



1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aim

- To determine the proportion of children and adults with NF1 associated plexiform neurofibromas who will have an objective response to binimetinib defined as a 20% or greater decrease in tumor volume reduction by 12 courses.

1.2 Secondary Aims

- To evaluate the feasibility and toxicity of protracted binimetinib administration in this patient population.

1.3 Exploratory Aims

- To evaluate the effect of binimetinib on quality of life and pain.
- To validate the NRS-11, which has been adapted to assess pain intensity in individuals with NF1 and plexiform neurofibromas, for use in this patient population
- To characterize the activity of binimetinib on plasma cytokines and growth factors
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- To obtain blood and tumor tissue to be stored for future PN biological studies.
- To compare the volumetric assessment of PN response to therapy performed by two independent analysts using different volumetric analysis methods
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2.0 BACKGROUND AND RATIONALE

2.1 Neurofibromatosis Type 1 and Plexiform Neurofibromas

Neurofibromatosis type 1 (NF1) is a common autosomal dominant, genetic disorder with an incidence of 1:3000. NF1 is caused by a mutation in the NF1 tumor suppressor gene. NF1 is characterized by diverse, progressive cutaneous, neurological, skeletal, and neoplastic manifestations. Patients with NF1 have an increased risk of developing tumors of the central and peripheral nervous system including plexiform neurofibromas, which are benign peripheral nerve tumors. These tumors may cause significant morbidity including disfigurement and pain and may be life-threatening when they compress vital structures such as the spinal cord, trachea, and great vessels.

The only proven treatment for plexiform neurofibromas is surgical removal; however, this is rarely achieved because of the infiltrative nature of the tumor and the high-risk of neurological morbidity. There is no currently accepted effective drug therapy for plexiform neurofibromas. A number of medical treatments including thalidomide 9, cis-retinoic acid, interferon alpha 2b [2, 3], methotrexate and vinblastine, PEG interferon alpha-2b [4], the farnesyltransferase inhibitor tipifarnib (R115777) [5, 6], the antifibrotic agent pirfenidone [7, 8], the RAF kinase inhibitor Sorafenib [9], the cKIT inhibitor Gleevec [10], and the mTOR inhibitor Sirolimus have been evaluated but to date no medical treatment has reliably resulted in objective radiographic response of plexiform neurofibromas.

NF1 encodes the tumor suppressor neurofibromin, a Ras-GTPase activating protein that converts active Ras-GTP to inactive Ras-GDP [11, 12]. In benign and malignant peripheral nerve tumors, cells acquire somatic inactivation of a second NF1 allele, with loss of neurofibromin function resulting in elevated levels of active Ras-GTP [13]. Aberrant Ras signaling promotes tumor cell proliferation and survival [14]. Research focused on the biology of NF1 and pathogenesis of plexiform neurofibroma has identified potential therapeutic targets including Ras itself, Ras effectors, tumor microenvironment, and angiogenesis.

The PI3K/AKT/mTOR pathway, known to regulate cellular proliferation and survival, has been implicated in NF1 tumorigenesis [15]. However, preclinical studies in Nf1fl/fl;Dhh-Cre mice with PN treated with the rapamycin analog everolimus failed to block tumor growth, and the multikinase inhibitor sorafenib affected only 5 of 9 (56%) mice [9]. A Phase I trial of sorafenib failed due to dose limiting toxicities of pain and mood changes in NF1 patients with PN [16], and a phase II trial of rapamycin in plexiform neurofibromas was not effective in shrinking non-progressive plexiforms [17], and modestly prolonged the time to progression of progressive PN.

To date, no chemotherapeutic approach blocking any molecular target, including tyrosine kinases upstream of Ras, Ras itself, or Ras effectors has consistently prevented or arrested neurofibroma formation [18] .

A recent preclinical study by Jessen and colleagues represents the most dramatic result described to date for treatment of neurofibroma bearing mice [19]. In this study, cross-species transcriptome analyses of mouse and human neurofibromas and MPNSTs identified global negative feedback of genes that regulate Ras-Raf-MEK-ERK signaling in both species. Nonetheless, activation of ERK was sustained in mouse and human neurofibromas. PD0325901, a highly selective pharmacological inhibitor of MEK, was used to test whether sustained Ras-Raf-MEK-ERK signaling contributed to neurofibroma growth in the Nf1fl/fl;Dhh-cre mouse model [19]. PD0325901 demonstrated remarkable effect, with reduction of aberrantly proliferating cells in neurofibromas and shrinkage of neurofibroma tumors in 39/49 (>80%) of mice tested. PD0325901 also caused effects on tumor vasculature. Similarly, dramatic responses to PD0325901 were seen in a murine model of juvenile myelomonocytic leukemia (JMML) characterized by homozygous Nf1 inactivation [19]. Together, these data provide a strong rationale for targeting MEK in the treatment of NF1-associated neoplasms in patients.

Indeed, results from a recent Phase I trial of MEK inhibitor AZD6244 in children with plexiform neurofibromas demonstrates an early efficacy signal in this population with partial responses (PN volume decrease of ≥ 20%) in 71% of evaluable subjects [20]. A Phase II trial is currently ongoing that also includes functional outcome measures as part of the overall benefit analysis of this therapeutic approach (NCT02407405).

2.2 Binimetinib

Binimetinib (also known as MEK162 or ARRY-438162) is a potent, selective, allosteric small-molecule inhibitor of MEK that is uncompetitive with adenosine triphosphate (ATP). Binimetinib is currently being evaluated clinically in healthy subjects and in cancer patients.

In cell-free systems, binimetinib inhibits MEK1/2 with an IC50 of 12 nM. *In vitro*, binimetinib potently inhibits MEK dependent phosphorylation of ERK in human BRAF-mutant melanoma cell lines, including A375, IGR-1, IGR-39, Colo-800, MDA-MB-435S, RPMI-7951, UACC-62, and WM-115, as well as NRAS-mutant melanoma lines, such as IPC-298, Hs944.T, MEL-JUSO, SK-MEL-2, and SK-MEL-30. *In vivo*, binimetinib treatment results in dose- and time-dependent inhibition of phosphorylation of ERK in

the HT-29 human colorectal carcinoma xenograft. Similarly, levels of both pERK and DUSP6 mRNA (a target gene of phosphorylated ERK) are reduced in the A375 melanoma xenograft. Lastly, in *ex vivo* experiments, binimetinib reduces pERK levels in human whole blood cells (Investigator Brochure (IB) version 12.1).

2.3 Pharmacokinetics and Dosing of binimetinib

The oral bioavailability (F) in rat and monkey absorption, distribution, metabolism and excretion (ADME) studies is moderate (48%) and similar to the fraction absorbed (based on radioactivity excreted) suggesting a minimal first pass effect. Binimetinib shows a low total systemic plasma clearance and volume of distribution at steady state after intravenous (IV) administration. Mean plasma terminal half-life (T1/2) ranges from 2 to 9 hours in preclinical species.

In vitro experiments indicate that binimetinib has moderate to high membrane permeability and is a substrate of P-gp and BCRP.

Binimetinib is highly bound to plasma proteins (humans: 97.2%).

Binimetinib is more distributed in plasma than blood. The blood-to-plasma concentration ratios of binimetinib ranges from 0.652 to 0.994 in the species tested. In humans, the blood-to-plasma ratio is 0.718.

[^{14}C]-binimetinib-derived radioactivity is absorbed and widely distributed to tissues in both pigmented rats and albino rats following a single oral (PO) dose.

With multiple routes of metabolism, binimetinib is metabolized primarily by glucuronidation pathways (mainly via UGT1A1, 1A3 and 1A9) and to a lesser extent by oxidation pathways (mainly via CYP1A2 and 2C19). UGT1A1 was shown to be the major contributor (90%) to the formation of the direct glucuronide.

Following IV dosing in the rat, fecal and urinary excretion accounts for 46% and 45% of total radioactivity, respectively. Approximately 15% of binimetinib is excreted unchanged in urine and 16 % in feces. Total recovery of radioactivity in the excreta of monkey is 99% and 85% following PO and IV dosing, respectively. The most abundant drug-related components in monkey excreta included binimetinib, the 2 glucuronides and the amide.

In vitro, binimetinib reversibly inhibits CYP2B6 and is a weak reversible inhibitor of CYP1A2 and CYP2C9. Binimetinib is not considered a time-dependent inhibitor of CYP1A2, CYP2C9, CYP2D6 or CYP3A4/5. Binimetinib induces CYP3A, but this was not confirmed in a human DDI study (please see Investigator's Brochure).

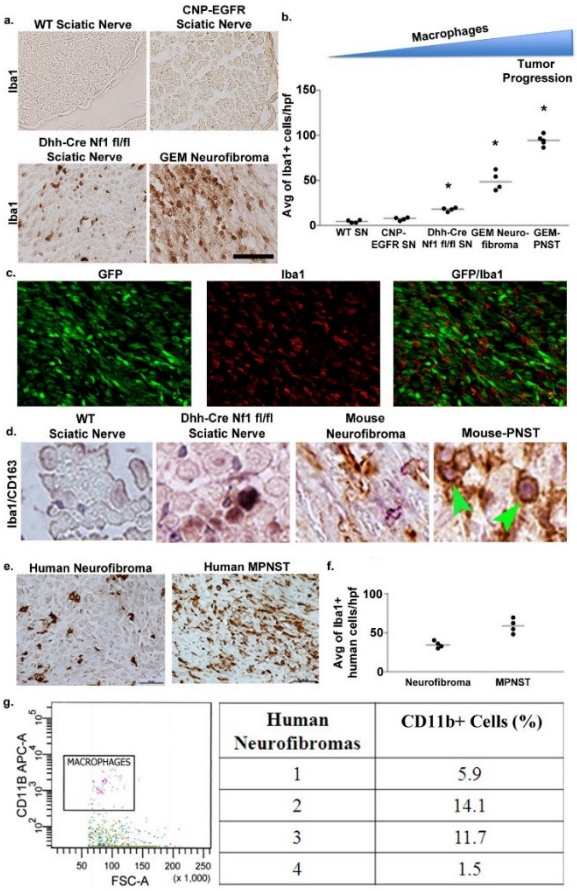
Preliminary findings from a phase I portion of a phase I/II study of Binimetinib for children with low grade glioma (NCT02285439) are as follows: 19 children were enrolled on the phase I study, 18 of whom were DLT-evaluable. Three DLT-evaluable participants were enrolled and treated at each dose level from 1 through 4, and 6 DLT-evaluable participants at dose level 5 (32mg/m2/dose bid). No DLTs were observed during the DLT evaluation period in any participant. Preliminary analysis of initial pharmacokinetic data showed that the pediatric AUC and Cmax values at 32mg/m2/dose were similar to corresponding values in adult patients at the adult RP2D of 45 mg/dose bid. Based on these findings, the convened study committee determined 32mg/m2/dose bid to be the pediatric RP2D for the phase II portion of the low-grade glioma study.

2.4 Patient Reported Outcomes

Although PN growth can be variable and unpredictable, we know that early adolescence through young adulthood is a critical period for the accelerated growth of neurofibromas [4, 21]. In fact, neurofibromas can continue to grow into later adulthood and continue to significantly impact health-related quality of life (HRQOL) of individuals with NF1. Participants of NF1 clinical trials traditionally undergo objective imaging and examination follow up to monitor changes in clinical symptoms. Volumetric MRI has become a standard imaging modality to measure tumor response. However, defining response solely from imaging of tumor size is difficult, as neurofibromas have irregular shapes and may be fibrotic; thus, with treatment, imaging may not always show a significant decrease in tumor size despite a clinically significant improvement in symptoms. Patients with little tumor shrinkage have reported large improvements in functioning and well-being that could be measured with a NF1-specific HRQOL instrument [21, 22]. As such, NF1-specific HRQOL instruments have been incorporated in all NF Consortium plexiform neurofibroma trials. With the above logic in mind, we include adults in this clinical trial, with complementary endpoints of imaging response measured via volumetric MRI and validated NF1-specific HRQOL assessments (including pain assessments) as well as functional assessments of vision and motor function to enhance our ability to assess the utility of this new therapy paradigm.

2.5 Cytokine and Plexiform Neurofibroma

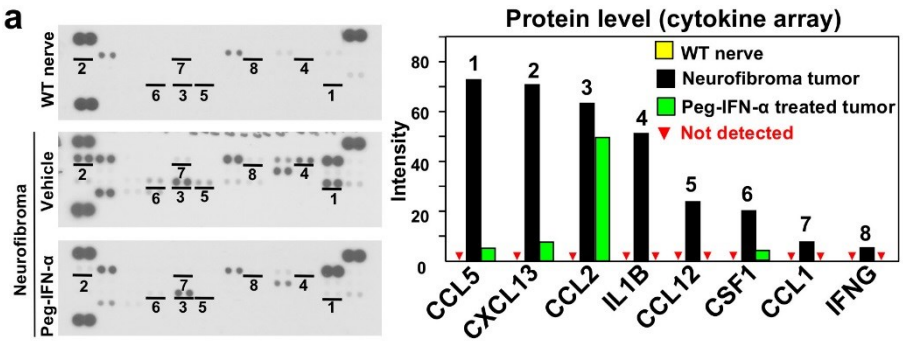
Neurofibromas are complex tumors, containing not only Schwann cells and vasculature, but also hematopoietic cells including mast cells and macrophages. Recent data from the Ratner lab [23] indicate that roughly 30% of neurofibroma cells are macrophages. Based on this finding, we hypothesized that patient plasma contains growth factors/cytokines that attract these hematopoietic cells to neurofibromas. Such factors could be biomarkers of tumor burden and/or play roles in tumor growth, or response to therapy.



Human Neurofibromas	CD11b+ Cells (%)
1	5.9
2	14.1
3	11.7
4	1.5

Figure 1. ***Iba1+ macrophages in nerve and neurofibroma***. **(A)** Staining of paraffin sections with anti-Iba1 (brown) to mark macrophages. GEM genetically engineered mouse model. **(B)** Quantification of Iba1+ macrophages per high-powered field (hfp) (*p=0.001). Averages from individual mice are shown as individual dots. **(C)** Staining of Dhh-Cre; $Nf1^{fl/fl}$ sciatic nerve sections with anti-Iba1 to mark macrophages (red) and with anti-GFP to mark Schwann cells (green). **(D)** M2 macrophages are rare in Nf1-deficient nerves and tumors. Iba1 (brown) and CD163 (purple) in paraffin sections. Green arrowheads show rare double-labeled cells. In all other cases, cells are single labeled. **(E)** Staining of human plexiform neurofibroma (PNF) and malignant peripheral nerve sheath tumor (MPNST) paraffin sections with anti-Iba1 (brown) to mark macrophages. **(F)** Average numbers of Iba1+ cells in human neurofibroma and MPNST. **(G)** FACS

shows populations of CD11b cells (macrophages) isolated from human neurofibromas after in vitro culture; table presents quantification. Scale bar in Fig. 1.



Cytokines, chemokines, and growth factors mediate and regulate immunity and inflammation, which are an important component of the tumor microenvironment contributing to tumor initiation and growth. These factors may also be useful as biomarkers tumor response. The literature shows that multiple cytokines/growth factors are altered in subjects with plexiform neurofibromas. In our mouse model of NF1, our unpublished filter-based multiplex detection data shows that the cytokine levels are associated with tumor progression and responsiveness to MEK inhibition (not shown) and PEG-Interferon therapy in mice (Choi et al., submitted; Figure 2). **Pro-inflammatory cytokines in Neurofibroma** (a) Left panel: Pro-inflammatory cytokines are at low levels in wild-type nerve (top), show increased protein levels in neurofibroma (middle), and are reduced after treatment of neurofibroma with PEGylated IFN-α2b (bottom). Right panel: Relative intensity, reflecting comparative levels of expression for each protein, after the intensity of pixels was averaged and plotted.

Multiplex analysis permits the characterization of groups of cytokines/chemokines/growth factors in a single sample. Because cytokines operate in integrated networks, a more complete understanding will be gained as multiple cytokines can be examined for patterns of response associated with response to therapy.

The Luminex is a high throughput multiplexing suspension array system. It enables the simultaneous quantitation of groups of cytokines and mediators in a single human, sample (i.e. serum/plasma), using magnetic beads that are coupled to antibodies. This assay is particularly useful for measurement of mediators/cytokines in small samples.

This Plasma biomarker panel (cytokine/chemokine/growth factor) simultaneously quantifies thirty soluble cytokines and growth factors including: VEGF, TGF-β, PDGF-AB/BB, MCP-1, MIP1α, MIP1β, TNFα, GM-CSF, EGF, IL-13, IL-1rα, IL-10, IL-4, IL6, FGF-2, and RANTES. The assay has large a dynamic range of 0.2 -10,000 pg. The multiplex assay will be performed according to the manufacturer's protocol (Millipore Milliplex, Billerica, MA) and analyzed on a Luminex 100 cytometer with StarStation software (Luminex Corp, Austin, TX).

2.6 Volumetric Assessment of Plexiform Neurofibroma Size

PNs are complex-shaped and slow-growing peripheral nerve sheath tumors that are challenging to measure by simple linear measurements. Automated volumetric MRI analysis can sensitively and reproducibly detect small changes in PN size, and it has become the measurement method of choice to determine tumor response and time to disease progression in recent clinical trials. There are two volume segmentation methods optimized for PN measurement, MEDx developed at the National Cancer Institute (NCI) and 3DQI used by the Tumor Imaging Metrics Core at Massachusetts General Hospital (MGH). The NF Clinical Trials Consortium has utilized MEDx in all its PN trials. In a small, retrospective study volume measurements and volume changes over time were compared using these two different methods and found good agreement. The goal is to prospectively evaluate a larger cohort of clinical trial

participants to further define the level of agreement in volume measurements, disease status classifications, and objective response rates between MEDx and 3DQI.

2.7 Overview of Proposed Study

This phase II open label study will evaluate children ≥ 1 year of age and adults with neurofibromatosis type-1 (NF1) and plexiform neurofibromas treated with the MEK inhibitor binimetinib. The primary aim of the study will be to assess quantitative radiographic response in a target lesion. Initially, adult subjects (Stratum A) received binimetinib by mouth on a BID dosing schedule of 45mg/dose (the established adult RP2D). Because 4 of the first 11 enrolled subjects chose to remove themselves from study for "intolerable" skin adverse events (AE's) at the 45 mg BID dosing, the starting dose for the adult stratum is reduced to 30 mg BID in Protocol Version 2.0. Pediatric subjects (Stratum B) will be treated on a BID dosing schedule of 32 mg/m^2, which has been established as the pediatric RP2D by study NCT02285439; maximum dose will be 45mg/dose (which corresponds to the adult MTD). Given the difference in the clinical behavior of plexiform neurofibromas in the adult and pediatric setting, Stratum A and B will be analyzed independently. Each course is 4 weeks duration. Subjects who have a 20% or greater reduction in target tumor volume at the end of 12 courses can continue on therapy for up to an additional year (maximum of 24 total courses). However, subjects who do not achieve at least 20% reduction in volume of the target tumor after 12 courses will be considered treatment failures and taken off study. Of note, subjects may begin course 13 while central MRI tumor response evaluations are pending.

Subjects entered on the trial will be carefully monitored for the development of binimetinib associated toxicities. Stopping and modification rules for toxicity are outlined in section 8.

Since plexiform neurofibromas may significantly impact the lives of patients with NF1[24], this study will evaluate the effects of the disease and treatment with binimetinib on the quality of life (QOL) and pain of subjects. Involvement in this part of the study will be required.

For subjects who respond to binimetinib (≥20% tumor volume reduction of target lesion by 12 courses), an MRI scan of the target lesion must be performed at 4 and 12 months after stopping drug (as long as the subject is still on protocol) in order to determine whether response is maintained post-therapy. These studies will not be requested from subjects who experience disease progression (≥ 20% tumor growth or increase of target lesion) while on study drug.

As of Protocol version 3.0, study participants who completed 24 courses of binimetinib and discontinued treatment at that time per protocol, but subsequently have tumor progression (>20% increase in volume) within one year of therapy completion, will be allowed to restart binimetinib at the same dose they were receiving at the end of the initial 24 courses, on the Re-Treatment Arm of the study.

3.0 SCREENING AND STUDY ENROLLMENT PROCEDURES

The NF Operations Center should be contacted to ensure availability of a treatment slot.

3.1 Informed Consent/Assent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject or the subject's parent(s) or guardian if the subject is a child, and a signed informed consent and assent will be obtained according to institutional and federal guidelines. In addition, study participants should sign the institution's HIPAA Consent if not already included in the study consent document.

3.2 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent. Documentation of the informed consent for screening will be maintained in the subject's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

Before the subject can be enrolled, the responsible institutional investigator must sign and date the completed eligibility checklist. The completed eligibility checklist and de-identified supporting clinical documentation should be scanned and emailed for review and signed off by the Study PI and/or Study Co-PI and the NF Operations Center to confirm eligibility prior to subject enrollment.

3.3 Study Enrollment

Subjects may be enrolled on the study once all eligibility requirements for the study have been met and a treatment number for subject treatment has been confirmed by the NF Consortium Operations Center as well as either the study chair or study co-chair. Subjects who give informed consent for the protocol to undergo screening for eligibility are not considered enrolled and should not be registered until the screening is completed and they are determined to meet all eligibility criteria. Treatment must start within 14 days of enrollment. **Subjects must not receive any protocol therapy prior to enrollment.**

4.0 PATIENT ELIGIBILITY

All studies to determine eligibility must be performed within 2 weeks prior to enrollment unless otherwise noted. All clinical and laboratory data required for determining eligibility of a subject enrolled on this trial must be available in the subject's medical or research record which will serve as the source document for verification at the time of audit/monitoring.

4.1 Inclusion Criteria for subjects at time of initial enrollment:

4.1.1 All subjects must have EITHER the clinical diagnosis of NF1 using the NIH Consensus Conference criteria (see Appendix III) OR have a constitutional NF1 mutation documented in a CLIA/CAP certified lab.

4.1.2 Subjects must have plexiform neurofibroma(s) that are progressive (see section 4.1.2.1) **OR** are causing significant morbidity, such as (but not limited to) head and neck lesions that are compromising the airway or great vessels, brachial or lumbar plexus lesions that are causing nerve compression and loss of function, lesions causing major deformity (e.g., orbital lesions) or are significantly disfiguring (see section 4.1.2.2), lesions of the extremity that cause limb hypertrophy or loss of function, and painful lesions. Subjects with paraspinal plexiform neurofibromas will be eligible for this trial. Histologic confirmation of tumor is not necessary in the presence of consistent clinical and radiographic findings but should be considered if malignant degeneration of a plexiform neurofibroma is clinically suspected.

4.1.2.1 For subjects enrolled for tumor progression, progression is defined as:

- Presence of new plexiform neurofibroma on MRI or CT (documented by comparison with prior MRI or CT), OR

- A measurable increase in plexiform neurofibroma size (≥ 20% increase in the volume, or a ≥ 13% increase in the product of the two longest perpendicular diameters, or a ≥ 6% increase in the longest diameter) documented by comparison of two scans (MRI or CT) in the time period of approximately 18 months or less prior to evaluation for this study.

4.1.2.2 For subjects enrolled for a "major deformity" or "significantly disfiguring" tumor, eligible tumors will be limited to tumors of the head & neck or those on other areas of the body that are unable to be concealed by standard garments. In order to enroll a plexiform neurofibroma for these indications, the Study Chair and/or Co-Chair must be contacted to review subject eligibility prior to enrollment.

4.1.3 Measurable disease: Subjects must have measurable plexiform neurofibroma(s) amenable to volumetric MRI analysis. For the purpose of this study, the target lesion must be seen on at least 3 consecutive MRI slices and the field of view must contain the entire tumor of interest. Tumors must be at least 3 mL in volume (most PNs 3 cm in longest diameter will meet this criteria). If the tumor is <3 cm in longest diameter, the subject may still be eligible. **Central review of the MRI of the target plexiform is required prior to enrollment to ensure that the tumor is measurable and amenable to volumetric analysis.** After consenting, please send the images on a CD along with the form in Appendix IVa to Eva Dombi (see Appendix IVa for address). Central review will take 3-7 days (please plan accordingly).

4.1.4. Age:

4.1.4.1. Stratum A: Subjects must be ≥ 18 years of age at the time of enrollment.

4.1.4.2. Stratum B: Subjects must be ≥ 1 year and < 18 years of age at the time of study enrollment.

4.1.5. Performance Level:

Karnofsky or Lansky ≥ 50% (Appendix II). Note: Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

4.1.6. Prior Therapy:

Subjects are only eligible if complete resection of a plexiform neurofibroma with acceptable morbidity is not feasible, or if a subject with surgical option refuses surgery.

Subjects who underwent surgery for a progressive plexiform neurofibroma will be eligible to enter the study after the surgery, provided the plexiform neurofibroma was incompletely resected and is evaluable by volumetric analysis (see section 4.1.3).

Subjects may have been previously treated for a plexiform neurofibroma or other tumor/malignancy but must have fully recovered from the acute toxic effects of all prior chemotherapy or radiotherapy prior to entering this study.

a. Myelosuppressive chemotherapy: Must not have received within 3 weeks of entry onto this study.
b. Hematopoietic growth factors: At least 7 days since the completion of therapy with a growth factor that supports platelet, red or white cell number or function.
c. Biologic (anti-neoplastic agent): At least 4 weeks since the completion of therapy with a biologic agent. For agents that have known adverse events occurring beyond 14 days after administration, this period must be extended beyond the time during which adverse events are

known to occur. These subjects must be discussed with the Study Chair on a case-by-case basis.

d. Investigational Drugs: Subjects must not have received an investigational drug within 4 weeks.
e. Steroids: Subjects with endocrine deficiencies are allowed to receive physiologic or stress doses of steroids if necessary.
f. Radiation: ≥ 6 months from involved field radiation to index plexiform neurofibroma(s); ≥ 6 weeks must have elapsed if subject has received radiation to areas outside index plexiform neurofibroma(s). Subjects who have received radiation to the orbit at any time are excluded.
g. Surgery: At least 3 weeks since undergoing any major surgery and must be recovered from effects of surgery.

4.1.7. Organ Function Requirements

4.1.7.1. Adequate bone marrow function defined as:
- Peripheral absolute neutrophil count (ANC) ≥ 1500/μL **and**
- Platelet count ≥ 100,000/μL (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment) **and**
- Hemoglobin ≥ 10.0 gm/dL without transfusions.

4.1.7.2. Adequate renal function defined as: maximum serum creatinine based on age/gender as per institutional standards OR a creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m^2

4.1.7.3. Adequate liver function defined as:
- Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x upper limit of normal (ULN) for age, **and**
- SGPT (ALT) ≤ 2.5 x upper limit of normal (ULN) for age, **and**
- Serum albumin ≥ 2 g/dL.

4.1.7.4. The effects of binimetinib on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and 3 months after completion of binimetinib administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

4.1.7.5. Adequate cardiac function defined as: left ventricular fractions (LVEF) ≥50% as determined by a multigated acquisition (MUGA) scan or echocardiogram, **and** QTc interval ≤480 ms.

4.1.7.6. Ability to comply with follow up procedures.

4.1.7.7. Negative urine or serum β-HCG test (females of childbearing potential only).

4.1.7.8. Patients must have a weight of >10 kg at enrollment

4.2 Exclusion Criteria for all subjects (including the Re-Treatment Arm)

4.2.1 Exclusion Criteria

- Chronic treatment with systemic steroids or another immunosuppressive agent. Subjects with endocrine deficiencies are allowed to receive physiologic or stress doses of steroids if necessary.
- Evidence of an active optic glioma or other low-grade glioma, requiring treatment with chemotherapy or radiation therapy. Subjects not requiring treatment are eligible for this protocol.
- Patients with malignant glioma, malignant peripheral nerve sheath tumor, or other malignancy requiring treatment in the last 12 months.
- Subjects who have received radiation to the orbit at any time previously.
- Ophthalmologic conditions:
  - Current or past history of central serous retinopathy
  - Current or past history of retinal vein occlusion
  - Known intraocular pressure (IOP) > 21 mmHg (or ULN adjusted by age) or uncontrolled glaucoma (irrespective of IOP). Patients with known glaucoma and increased IOP who do not have meaningful vision (light perception only or no light perception) and are not experiencing pain related to the glaucoma, may be eligible after discussion with the study chair. Patients with orbital plexiform neurofibromas should have IOP measured prior to enrollment.
  - Subjects with any other significant abnormality on ophthalmic examination should be discussed with the Study Chair for potential eligibility.
  - Ophthalmological findings secondary to long-standing optic pathway glioma (such as visual loss, optic nerve pallor or strabismus) or long-standing orbito-temporal PN (such as visual loss, strabismus) will NOT be considered a significant abnormality for the purposes of the study.
- Uncontrolled arterial hypertension despite medical treatment defined as CTCAE grade 3 or higher. Use age and gender appropriate normal values >95th percentile ULN for pediatric patients. See Appendix XVII.
- Impaired cardiovascular function or clinically significant cardiovascular diseases, including:
  - History of acute coronary syndromes (including myocardial infarction, unstable angina, coronary artery bypass grafting, coronary angioplasty, or stenting) <6 months prior to screening
  - Symptomatic chronic heart failure, history or current evidence of clinically significant cardiac arrhythmia and/or conduction abnormality <6 months prior to screening except atrial fibrillation and paroxysmal supraventricular tachycardia
- Other concurrent severe and/or uncontrolled medical disease, which could compromise participation in the study (e.g. uncontrolled diabetes, uncontrolled hypertension, severe infection, severe malnutrition, chronic liver or renal disease, active upper GI tract ulceration, congestive heart failure, etc.)
- Subjects who have an uncontrolled infection.
- Known positive serology for HIV (human immunodeficiency virus), active hepatitis B, and/or active hepatitis C infection.
- Impairment of gastrointestinal function or gastrointestinal disease (e.g. ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection).

- History of Gilbert's syndrome or patients who are known to be homozygous for UGT1A1 (7/7).
- Patients who have neuromuscular disorders that are associated with elevated CK (e.g., inflammatory myopathies, muscular dystrophy, amyotrophic lateral sclerosis, spinal muscular atrophy)
- Subjects who are planning to embark on a new strenuous exercise regimen after first dose of study treatment. NB: muscular activities, such as strenuous exercise, that can result in significant increases in plasma CK levels should be avoided while on binimetinib treatment.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to binimetinib.
- Women who are pregnant or breast feeding.
- Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures, e.g., infection/inflammation, intestinal obstruction, unable to swallow, social/ psychological issues, etc.
- History of noncompliance to medical regimens.
- Subjects unwilling to or unable to comply with the protocol, or who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study.
- Prior treatment with a MEK inhibitor of any kind (not applicable for the Re-Treatment Arm).

4.3 Re-Treatment Arm

All studies to determine eligibility for Re-Treatment must be performed within 2 weeks prior to "enrollment" on the Re-Treatment arm except for MRI, ophthalmology, Echo, and EKG (which is required within 4 weeks prior to "enrollment" on the Re-Treatment Arm), unless otherwise indicated. Re-Treatment eligibility studies may be counted as the Re-Treatment baseline studies as long as there has been no concerning change in clinical status prior to starting study drug, except for pregnancy testing in females of childbearing age which must be negative within 7 days prior to re-starting binimetinib. Study drug must start within 14 days of "enrollment" on the Re-Treatment Arm.

4.3.1 Inclusion Criteria for Re-Treatment

- Participants who had a partial response to binimetinib, completed 24 courses of initial treatment and discontinued treatment at that time as per study protocol.
- Progression of the originally identified target PN (defined as ≥20% increase in tumor volume *compared with* post course 24 volume) within one year of stopping initial treatment as per central review.
- Must have recovered to grade 1 or less from any prior binimetinib-related toxicities.
- Performance Level: Karnofsky or Lansky ≥ 50% (Appendix II). Note: Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.
- Organ Function Requirements: Must meet all organ function requirements as per section 4.1.7.

4.3.2 Exclusion Criteria for Re-Treatment

- Participants who stopped binimetinib treatment for any other reason other than discontinuation after completion of 24 courses of initial treatment as per protocol.
- Treatment with tumor-directed therapy for the target PN or any other tumor since discontinuing binimetinib.

- Surgery on the target PN since discontinuing binimetinib.
- >13 months have elapsed since discontinuing binimetinib.
- All exclusion criteria in section 4.2.1 must be met except for prior treatment with a MEK inhibitor of any kind.

5.0 REQUIRED DATA

IRB Approvals

The University of Alabama at Birmingham's Office of Institutional Review Board for Human Use has offered to serve as the Lead IRB for all institutions via SMART IRB. Participating sites of this protocol are encouraged to utilize this infrastructure to expedite the review process. Participating sites will need to have an existing Master Common Reciprocal Institutional Review Board Authorization Agreement with "SMART IRB" to utilize the UAB IRB as their IRB of record. Sites that use the single IRB will rely on the UAB IRB approval so that all study sites have the same IRB determination, consent template(s), and expiration date.

Sites that choose not to use the UAB IRB as IRB of record must provide the NF Consortium Operations Center with a copy of the initial IRB protocol approval and the yearly IRB continuing reviews. The NF Clinical Trial Consortium Regulatory Specialist will submit these to the USAMRMC ORP HRPO on behalf of each site and maintain a master regulatory file. Registration will be halted at a participating institution if a current continuing approval is not on file at the NF Consortium Operations Center.

Each participating institution is required to maintain a current MPA or FWA in order to participate in government-sponsored Group research. The files will be copied or made available for review by authorized persons as required for conduct of this trial.

Amendments and Consents

The PI from each participating institution will provide the NF Consortium Operations Center with a copy of IRB approval of all amendments to the protocol or consent. The Regulatory Specialist representing the NF Consortium Operations Center will provide these institutional reviews to the USAMRMC ORP HRPO on behalf of each site and maintain a master regulatory file.

As this trial receives funding by the US Army, substantive amendments to the research protocol and any amendments that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation and site distribution. The USAMRMC ORP HRPO defines a substantive amendment as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

Data Collection and Toxicity Reporting

The trial is being conducted by the NF Consortium in collaboration with PNOC. Case report forms developed by the NF Consortium Operations Center and Protocol Chair will be used for submitting clinical data to the Operations Center. Data must be submitted to the Operations Center within two

weeks of completing each required evaluation while the subject is on study. Delinquent data delays will be reported to the NF Consortium's Site Evaluation Committee and the NF Operations Center.

The NCI POB will receive MRI studies electronically or on CD and completed worksheets for volumetric analysis. When sending the CD, the site must provide tracking # (FedEx or UPS) by email to Eva Dombi: dombie@mail.nih.gov.

Completed QOL and pain evaluations will be submitted in real-time by email to the NF Operations Center.

Cincinnati Children's Hospital Medical Center will receive cytokine samples along with completed worksheets as outlined in Appendix VII. Upon analysis of these studies, results will be transmitted to the NF Consortium Operations Center for entry into the study databases.

Biological specimens for banking will be handled and stored in the biorepository located in the Colket Translational Research Building at the Children's Hospital of Philadelphia (CHOP), Philadelphia, PA. The specimens will be under the management of the Center for Data Driven Discovery in Biomedicine (D3b) and the Biorepository Core (BioRC), according to their standard operating procedures (SOPs). Samples will be stored at -80°C. Biological specimens will be coded with a randomly generated study number prior to shipping to the biorepository in order to prevent identification of subjects and protect confidentiality. The study number will not be derived from or related to information about the subject and will not contain any elements of PHI. The specimen number will be entered into the NF Consortium Operations Center eDES, thus providing a link between the specimen and the study data. Specimens may be shared with outside investigators/laboratories, who may analyze (and store, if applicable) the samples. Shared specimens will be labeled only with a study number. Requests for samples will be reviewed by the NF108 Study Chairs and the NFCTC Biology Committee. If a subject withdraws consent to store samples after collection, existing samples in the biorepository will be destroyed; however, any samples already shared to an outside laboratory or other entity will not be destroyed and any data generated already from the samples will not be destroyed.

Representatives from the FDA, NF Consortium Quality Assurance Committee, University of Alabama at Birmingham's Institutional Review Board and the U.S. Army Medical Research and Material Command will have access to the data and research records as required. Independent research monitor and DSMB will also have access to data and research records as required.

Julia Bender will serve as the research monitor and will serve as a patient advocate and is independent of the clinical study team. She will oversee the progress of the protocol, especially issues of individual subject/patient management and safety. The research monitor is required to review all unanticipated problems involving risks to subjects or others, including all serious and unexpected adverse events associated with the protocol as defined in Section 10.0. She will be automatically notified by the data entry system as soon as any SAE is entered into the study system. The monitor provides an unbiased written report of the event and may request additional information if needed for her determination.

Handling of Research Samples

This study is conducted at NF and PNOC Consortium Sites and will be coordinated by the NF Consortium Operations Center. Sample labeling, collection and initial processing will be conducted as outlined in the study Section 9, as well as in Appendix VII. Blood samples for cytokine studies will be sent to Cincinnati Children's Hospital Medical Center for analysis. Samples will be stored in designated monitored freezers and will be identified by the protocol specific subject ID number. Once analyzed for the studies outlined in this protocol, any remaining samples will be stored by the Cincinnati lab until the

study is complete and the manuscript describing the study has been accepted for publication. Any use of samples not outlined in Section 9 or Appendix VII will require Study Leadership approval and prospective IRB review and approval. The study will remain open and status reported to the IRB until all samples have been analyzed, reported or destroyed. Unintentional loss or destruction of any samples will be reported to the IRB as part of annual continuing reviews. MRI studies of PN will be obtained as described in Appendix IV and will be sent on CD to the NCI POB. MRI studies will be loaded on one of three Sun Workstations, which are password protected and allow access only to Drs. Dombi, Widemann, or associate investigators on the trial. CDs will be stored in locked filing cabinets. Data results will be electronically submitted to the Operations Center for curation in the study database.

Data and Center Audits

The trial will be monitored by the data center during the course of the study and monitored on-site by the NF Consortium Operations Center as needed for compliance and safety. Monitors may visit participating sites and review case report forms and source documentation. Missing or spurious information and protocol deviations will be communicated in a report to the NF Consortium Operations Center. Protocol deviations, which may compromise the ability to safely administer study drug or to accurately determine study endpoints, will be included in the annual protocol review to the USAMRMC ORP HRPO, the FDA, medical monitor, NF Consortium IRB and the DSMB for this study.

All unexpected and serious adverse events will be forwarded to the Medical Monitor, the Study PI, FDA, the DSMB and the USAMRMC ORP HRPO by the NF Consortium Operations Center as defined in Section 10.0.

Volumetric MRI analysis performed by the NCI-POB will be used to determine disease progression, and subjects will not be removed from study based on 1-D or 2-D MRI measurements or based on clinical measurement of superficial lesions.

5.1 Tests and Observations

See section 9.0 and Appendix I. Unless otherwise noted, all tests to determine eligibility must be completed within 14 days of study enrollment.

5.2 Records to be Kept

Subject Registration and Case Report Forms

Subjects will be registered with the NF Consortium Operations Center via the electronic data entry system (eDES). Electronic and paper Case Report Forms will be developed by the Consortium Operations Center and Protocol PI. All data must be entered into the eDES. Sites have the option of maintaining a printed copy of completed forms in the subject's study binder that have been initialed and dated by the study team member entering the data into the paper case report form.

Subjects must not be identified by name on any study documents that are sent off site to any agency. The Subject Identification Number received upon data entry registration and placed on all case report forms and regulatory documents will identify subjects.

All data entered on a paper CRF must be legibly recorded in indelible ink or typed. A correction should be made by striking through the incorrect entry with a single line and entering the correct information adjacent to it. The correction must be initialed and dated by the investigator or designated qualified

individual. Any requested information that is not obtained as specified in the protocol should have an explanation noted on the CRF as to why the required information was not obtained. The electronic CRF provides audit trail showing when data is revised.

5.3 Role of Data Management

5.3.1 Instructions concerning the recording of study data on CRFs will be provided by the NF Operations Center.

5.3.2 It is the responsibility of the NF Operations Center to assure the quality of data for this study in partnership with the study investigators. This role extends from protocol development to generation of the final study database.

6.0 TREATMENT PROGRAM

6.1 Agent Information/ binimetinib

6.1.1 Source and Pharmacology:

Absorption and distribution: Study [CMEK162A2102] investigated the absorption, distribution, metabolism, and elimination (ADME) of a single oral dose of 45 mg of [^{14}C]-binimetinib in healthy male subjects. The overall recovery of radioactivity in the excreta for all six subjects was 93.6 ± 3.27%, indicating that good mass balance was achieved. A lower limit on the extent of oral absorption for binimetinib in this study can be set at ~ 50% based on the percentage of radioactive dose recovered in the excreta. However, the actual value could be greater if a portion of the unchanged binimetinib observed in the feces was absorbed and then either excreted in bile, intestinally secreted, or metabolized to a glucuronide that was then hydrolyzed back to binimetinib in the gut. The mean blood to plasma concentration ratio for total radioactivity was comparable to the mean in vitro value for [^{14}C] binimetinib (0.63 versus 0.72). On average, ~60% of the circulating radioactivity AUC in plasma was attributable to binimetinib.

Food effects: Food-effect clinical studies have shown influence of food on PK of binimetinib is mild and not clinically relevant; therefore, binimetinib can be taken with food.

Pharmacokinetics and Metabolism:

In animals, exposure (AUC) and C_{max} generally increased in a dose proportional manner. The plasma clearance is low (range: ~2 to 8 mL/min/kg) and the mean plasma half-life values ranges from 2 to 9 hours. Binimetinib has moderate membrane permeability.

Binimetinib exhibited high plasma protein binding in vitro (> 96%, except dog 84%) and is predicted to have good stability with respect to hepatic metabolism. Nonclinical in vitro and in vivo data indicated that binimetinib is metabolized by multiple routes but primarily by glucuronidation pathways (mainly via UGT1A1, 1A3 and 1A9) and to a lesser extent by oxidation pathways (mainly via CYP1A2 and 2C19). UGT1A1 is considered the primary enzyme to mediate glucuronidation of binimetinib. The formation of active metabolite AR00426032 is mediated primarily by CYP1A2 with minor contribution by 2C19 and 2B6.

Excretion of [^{14}C] binimetinib in rats was split between the fecal and urinary routes. The predominant route of excretion in monkey was urinary. Binimetinib was mainly eliminated through glucuronidation and parent drug excretion. The major circulating component in rat and monkey plasma was binimetinib. The direct glucuronide was also observed in monkey plasma.

Binimetinib is a potent reversible inhibitor of CYP2B6. In vitro evidence also suggests that binimetinib could induce CYP3A.

Pre-clinical studies in mice support that >50% inhibition in pERK in HT-29 tumors for 24 hours is optimal for significant tumor growth inhibition. This degree of sustained pERK inhibition was best achieved with BID dosing at lower doses (< 10 mg/kg/dose) or QD dosing at higher doses (> 30 mg/kg/dose). Given that little to no drug was apparent in plasma at 24 hours post-dose (at low doses) in the pre-clinical xenograft studies, a BID dosing regimen was considered to be the most reliable murine schedule to maintain plasma concentrations for optimal efficacy. Therefore, a BID dosing regimen (i.e., 45 mg) should provide sufficient exposure with acceptable safety and adequate continuous pharmacological inhibition of the primary target.

In healthy subjects and patients with rheumatoid arthritis, the analysis of PK data was done using model independent (NCA) methods only. These analyses suggested that absorption was rapid, that there was extensive distribution and that the apparent terminal half-life was short. Contrary to expectation, given the short half-life, accumulation greater than that predicted by linear PK was observed. Overall, when given BID, accumulation was around 1.5 to 1.7-fold. This apparent non-linearity in the PK of binimetinib may have been related to a combination of a relatively high LLOQ of the assay used (5 ng/mL) and the schedule of blood collection for PK used. In order to overcome these limitations and to fully characterize the PK of binimetinib in cancer patients a model-based analysis was conducted using a population PK method. For this analysis, information from 130 subjects from two clinical studies, [CMEK162X2201] and [ARRAY-162-111], were used. Dose levels range from 30 mg BID to 80 mg BID.

Binimetinib PK was best described by a two-compartment disposition model with first-order elimination and first-order absorption with a lag time. The estimated between subject variability on apparent clearance (CL/F) was moderate (44%) and very high (~150%) for the apparent central volume of distribution (Vc/F) and apparent peripheral volume of distribution (Vp/F). The covariate effect of dose on relative bioavailability was identified as significant (at an $\alpha = 0.001$ significance level). Relative to the 45 mg dose, the bioavailability of the 30 mg dose level was 120%; while the relative bioavailability of 60 mg and 80 mg doses were 88% and 77%. This dose dependence was not seen using model independent methods but most likely represents either solubility limited absorption of binimetinib or the impact of P-gp or a combination of the two. Based on the PK parameters estimated in this analysis when administered BID, binimetinib steady state is reached around Day 15 and the accumulation is around 1.7-fold which is consistent with observation. For further information refer to the current binimetinib Investigator's Brochure. For further details on non-clinical pharmacokinetics and metabolism, please refer to the current binimetinib Investigator's Brochure.

Excretion: The human ADME [CMEK162A2102] study showed that after a single oral dose of 45 mg of [^{14}C] binimetinib, a mean of 62.3% of the radioactivity dose was eliminated in the feces while 31.4% was eliminated in the urine. In urine, 6.5% of the radioactivity dose was excreted as binimetinib. The estimated mean CLr value of 1.78 L/h was 6.3% of the total mean CL/F value of 28.2 L/h.

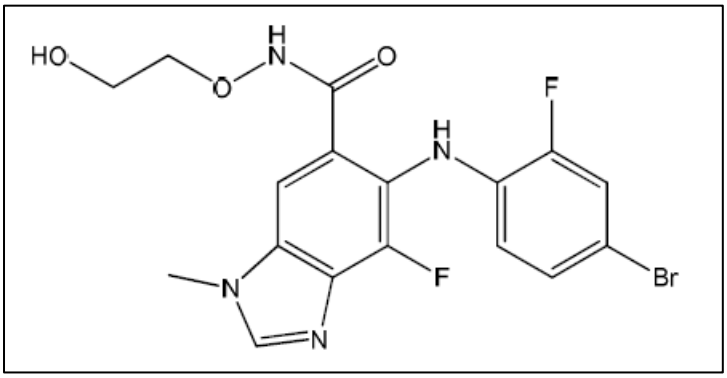
6.1.2 Formulation and Stability

Tablet information: Binimetinib drug product is supplied as film-coated tablets in dosage strength of 15 mg. The film-coated tablets consist of binimetinib, lactose monohydrate, microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, magnesium stearate and a commercial film coating. The tablets are yellow to dark yellow and capsule shaped.

Storage Conditions: Binimetinib film-coated tablets should not be stored above 25°C and should be protected from light. Tablets are packaged in plastic bottles acceptable for pharmaceutical use.

Relative molecular mass: 441.23

Chemical Structure:



CAS Name: 606143-89-9

Systematic (IUPAC) Name: 5-[(4-bromo-2-fluorophenyl)amino]-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1Hbenzimidazole-6-carboxamide.

Molecular Formula: $C_{17}H_{15}BrF_2N_4O_3$

Physical Description: White to light tan powder or white to slightly beige powder

6.1.3 Supplier

Array BioPharma, a wholly owned subsidiary of Pfizer Inc. will supply the drug in 15mg film-coated tablets. Pharmacy preparation instructions for liquid suspension can be found in Appendix XVIII.

6.1.4 Toxicities

Acute, subchronic, chronic and reproductive toxicity, genotoxicity and phototoxicity studies were completed to support the chronic administration of binimetinib to adult cancer patients. The toxic effects of MEK inhibitors in humans are similar to the toxic effects observed in monkeys. The toxic effects include gastro-intestinal intolerance and diarrhea, rash, central serous retinopathy (only seen in humans) and retinal vein occlusion (rarely seen in humans). *In vitro* and *in vivo* phototoxicity studies conducted in mice indicate that binimetinib has a very low risk of weak phototoxic potential at therapeutic doses. Furthermore, there has been no evidence of phototoxicity or photosensitivity in humans being treated with binimetinib for cancer or for rheumatoid arthritis. This includes 2555 patients and healthy volunteers who have received at least one dose of binimetinib and is based on data as of January 20, 2016. Given the embryo-lethal effects seen in rats and rabbits and the teratogenic effects seen in rabbits, binimetinib should not be used in pregnant women. Women of child-bearing potential must be advised to use highly effective contraception methods.

Data collected in the nonclinical and clinical programs, as well as class effects with MEK inhibitors overall, indicates that binimetinib exposure may be associated with rash-like events, gastrointestinal events (including diarrhea, nausea and vomiting, stomatitis/mucositis), reversible elevations of CK, edema, cardiac events, retinal events, and interstitial lung disease or non-infectious pneumonitis.

6.2 Treatment Overview

Binimetinib will be administered to adult subjects orally on a daily BID schedule, continuously without breaks, as a flat-fixed dose of 30 mg BID, and not by body weight or body surface area for patients ≥ 18 years of age. Subjects who have been enrolled prior to protocol version 2.0 and tolerate binimetinib at 45 mg PO BID can continue on that dosing regimen if the treating physician believes that this is in the best interest of the patient.

Binimetinib will be administered to children between 1 and 17 years orally as a BID schedule, continuously without breaks, at a dose of 32 mg/m^2 (maximum: 45mg dose) dose will be calculated based on body surface area and should be calculated approximately monthly; see Appendix XIX for liquid dosing and Appendix XX for tablet dosing.

The interval between dosing is 12 hours +/- 2 hours. If the patient is not able to take the medication within this time frame, that particular dose should be skipped and marked as missed on the drug diary. Patients will be supplied with a sufficient number of tablets, or liquid suspension, for the number of doses to be taken prior to the next scheduled visit. Tablets must be swallowed whole. In the pediatric stratum, a liquid suspension can be created, for ease of dosing and administration – please see Appendix XVIII. Patients in the pediatric stratum can take whole tablets if preferred and the dosage is within 10% of the recommended dose. Patients in the adult stratum may also use a liquid suspension if they are unable to swallow whole pills, following the dose reduction as in Section 8.2. Patients who require doses not within 10% of a whole tablet dose, may use a combination of tablets and liquid to achieve a dose, as the bioavailability of both formulations is excellent. For example, if the dose were 22 mg, and the volume of liquid was too challenging for the patient to take, they may take a 15 mg tablet + 7 mg liquid to achieve the dose. In addition, patients will be provided a dosing diary and should document in this diary each prescribed dose, and whether it was taken or not. Both a tablet and liquid diary should be used if formulations are being combine.

Each course is 4 weeks duration. Subjects who have a 20% or greater reduction in target tumor volume at the end of 12 courses can continue on therapy for up to an additional year (maximum of 24 total courses). Subjects who have not had at least 20% tumor shrinkage by volumetric analysis by the end of the 12th course will be removed from protocol therapy. Of note, subjects may begin course 13 while central review of tumor response is ongoing.

As of Protocol version 3.0, study participants who completed 24 courses of binimetinib and discontinued treatment at that time per protocol, but subsequently have progression of their target PN (>20% increase in volume) within one year of that time, will be allowed to restart binimetinib at the same dose they were receiving at the end of the initial 24 courses, on the Re-Treatment Arm of the study. Once they re-start treatment, participants may receive up to 24 courses of binimetinib, as long as the tumor does not progress (>20% increase in tumor volume *compared with* pre-Re-Treatment baseline). After 24 courses of re-treatment, for participants who are deriving benefit and for whom both the participant and treating physician agree that continued treatment with a MEK inhibitor is appropriate, such participants may continue to receive binimetinib via the study for up to 12 courses, while arrangements are being made to transition the participant to a MEK inhibitor (which may include binimetinib) off study. All efforts should

be made to transition the participant and perform end of study assessments as soon as possible after 24 courses of re-treatment.

Binimetinib can be taken with food.

Re-administration of dose: If a patient vomits at any time after dosing, the dose of study drug should **not** be re-administered. Doses of binimetinib that are omitted for AEs or any other reason should not be made up later in the day, or at the end of the dosing period.

6.3 Criteria for Starting Subsequent Courses

Unless the subject has developed toxicity requiring interruption or withdrawal from protocol therapy (see section 8.0), a course may be repeated every 28 days if the subject has at least stable disease and has again met laboratory parameters (when study evaluations are required) as defined in the eligibility section. **Of note, subjects may begin a course while central review of tumor response is ongoing.**

If weight falls below 10 kg during treatment, binimetinib should be held until weight recovers to ≥ 10 kg. If binimetinib is not resumed within 28 days, the patient should be removed from protocol therapy.

7.0 SUPPORTIVE CARE AND OTHER CONCOMITANT THERAPY

Subjects must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator. All medications taken within 30 days of screening should be recorded. If concomitant therapy must be added or changed, the reason and name of the drug/therapy should be recorded.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the subject are allowed, including drugs given prophylactically (e.g. anti-emetics), with the following exceptions:

- No other investigational therapy should be given to subjects
- No chronic treatment with systemic steroids or another immunosuppressive agent (see section 7.4) except for subjects with endocrine deficiencies who are allowed to receive physiologic or stress doses of steroids, if necessary.

Concurrent Anticancer Therapy

Concurrent cancer therapy, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered.

7.1 Supportive Care

Appropriate antibiotics, blood products, fluids, electrolytes, and general supportive care are to be used as necessary. Anti-emetics, **other than systemic steroids**, are allowed. Corticosteroids are permissible as premedication for blood product transfusions, or as treatment for an acute allergic reaction.

7.2 Growth Factors

Growth factors that support platelet or white cell number or function can only be administered for culture proven bacteremia, clinical sepsis, or invasive fungal infection with neutropenia. The Study Chair should be notified when growth factors are initiated.

7.3 Surgery

Subjects undergoing surgical procedures should have the study drug held for 2 weeks prior to the procedure, if feasible, and for 2 weeks after. Surgery to a non-target plexiform neurofibroma does not require removal from protocol therapy (i.e. subject can continue on therapy once he/she recovers from surgery, as described above).

7.4 Management of Hypertension, Skin Toxicity, and Nausea and Vomiting

Management of Hypertension
Patients that will receive binimetinib must have their blood pressure monitored as per the observation table in Appendix 1. If elevated blood pressure is observed, additional monitoring should occur per institutional guidelines.

The investigator is to educate the patient on the signs and symptoms of hypertension and use of the home blood pressure monitor. More frequent assessments during the study drug treatment period may also be performed at the discretion of the investigator and if medically indicated.

For patients monitoring their blood pressure at home, it is suggested to develop a patient diary to record their self-assessed blood pressure measurements and present the collected data to the investigator for evaluation and appropriate management per institutional guidelines. This diary must be maintained in the patient's source documentation.

Management of Skin Toxicity

Skin toxicity has been reported for binimetinib and has been encountered in this trial, often soon after starting medication. Therefore, it is recommended that patients are educated on the possibility of occurrence of these side effects prior to starting study treatment. Patient education as well as proper management of skin toxicity at the first sign is important. The following recommendations have been established to help minimize and manage skin toxicity and should be followed if deemed appropriate by the treating physician.

The following medications may be useful in managing these rashes:

Acneiform rash
- Doxycycline 100 mg BID (for patients >8 years of age weighing >45 kg)
- Doxycycline 2.2 mg/kg/dose BID (for patients >8 years of age weighing ≤45 kg)
- Clindamycin phosphate 1% lotion BID

Dry/flaky scalp
- Ketoconazole 2% shampoo

Eczematous rash
- Low potency topical steroid cream/ointment (not lotion) for face, axilla, groin
- Mid potency topical steroid cream/ointment (not lotion) for other locations

It is recommended that at the time of initial study drug dispense, that patients be sent home with prescriptions for doxycycline and clindamycin lotion, and that these be filled and started at the first sign of acneiform rash.

Management of Nausea and Vomiting

Because nausea and vomiting have been reported for binimetinib, it is recommended that patients are educated on the possibility of occurrence of these side effects prior to starting study treatment. Patient education as well as proper management of nausea and/or vomiting at the first sign is important. Clinical judgment and experience of the treating physician should guide the management plan of each patient. Patients experiencing nausea and/or vomiting CTCAE ≥ grade 1 will receive antiemetics at the discretion of the treating physician (as per local guideline). It is recommended that patients be provided a prescription for antiemetics and are instructed on the use of antiemetics on the first day of study drug treatment.

Dose interruption/reduction decisions for nausea and/or vomiting should be based on the CTCAE 4.03 grade of the toxicity and guidelines as listed in section 8.0.

7.5 Concomitant Medications

Permitted concomitant therapy requiring caution and/or action

Binimetinib potently inhibits CYP2B6 *in vitro* (Ki of 1.67 μM). Based on these findings, binimetinib may inhibit the metabolic clearance of co-medications metabolized by CYP2B6, if sufficiently high concentrations are achieved in vivo. At 45 mg BID, the maximum concentrations achieved in plasma are normally <1.5 μM so the risk of drug interaction is limited.

In vitro data showed that binimetinib is a substrate of P-gp. Binimetinib is also a substrate of BCRP. Thus, the use of drugs that are known to inhibit or induce P-gp and BCRP should be used with caution.

Binimetinib has been identified to be primarily metabolized by UGT1A1 *in vitro*. It is advised that inhibitors and inducers of UGT1A1 should be taken with caution when co-administered with MEK162. Patients should be closely monitored for the occurrence of adverse events. Please refer to Appendix VI for a list of these known drugs but this list may not be exhaustive.

Drugs with a conditional, possible, or known risk to induce Torsade de Pointes (TdP) should be used with caution. Patients receiving such medications must be carefully monitored for potentiating of toxicity due to any individual concomitant medication and may require dose titration of the drug substance. Investigators should use caution when prescribing co-medications, as clinical experience with these compounds in patients with cancer is often limited. **Please refer to Appendix VI for a list of permitted medications to be used with caution.**

8.0 MODIFICATIONS FOR ADVERSE EVENTS

Guidelines for Treatment Delay, Dose Reduction, or Study Drug Discontinuation for Toxicity
Subjects will be monitored continuously for AEs throughout the study and for 30 days after the last dose of study treatment and for any serious adverse event (SAE) assessed as related to study treatment or study procedures, even if the SAE occurs more than 30 days after the last dose of study treatment.

Subjects will be instructed to notify their physician immediately of any and all AEs. Subjects experiencing one or more AEs due to the study treatment may require a dosing delay or reduction(s) in their dose in order to continue with study treatment.

Adverse events will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) (v4.03).

Any subject experiencing a dose reduction toxicity (as described in table 8.6) that is possibly, probably, or definitely related to binimetinib should have the drug held until the toxicity improves to and remains stable at grade 1 or less (see section 8.2). In addition, **the site should notify the study chair sabine.mueller@ucsf.edu and co-chairs Fisherm@email.chop.edu and Alyssa.reddy@ucsf.edu along with the NF Operations Center nfcop@uab,edu immediately of any drug toxicity and/or dose reduction toxicity or study drug interruptions. Notifications should be by email.**

8.1 Grading of common adverse events for MEK inhibitors

Table 8-1 CTCAE grading for eye disorders

Grade	Description
1	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL
3	Severe or medically significant but not immediately sight threatening; Hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self-care ADL
4	Sight-threatening consequences; urgent intervention indicated; blindness (20/200 or worse) in the affected eye
ADL= activities of daily living	

Retinal Detachment should be graded according to the CTCAE version 4.03 as described below in Table 8-2.

Table 8-2 CTCAE grading for retinal detachment

Grade	Description
1	Asymptomatic
2	Exudative and visual acuity 20/40 or better
3	Rhegmatogenous or exudative detachment; operative intervention indicated; decline in vision (worse than 20/40*but better than 20/200*)
4	Blindness (20/200* or worse) in the affected eye

Uveitis should be graded according to the CTCAE version 4.03 as described below in Table 8-3.

Table 8-3 CTCAE grading for uveitis

Grade	Description
1	Asymptomatic; clinical or diagnostic observations only
2	Anterior uveitis; medical intervention indicated
3	Posterior or pan-uveitis
4	Blindness (20/200* or worse) in the affected eye
* Please refer to Snellen Equivalence (Visual Acuity Conversion Chart).	

Hand Foot Skin Reaction should be graded according to CTCAE version 4.03 as described below in Table 8-4.

Table 8-4 CTCAE grading of Hand foot skin reaction (HFSR)[a]

Grade	Description
1	Minimal skin changes or dermatitis (e.g., erythema, edema, numbness, dysesthesia, paresthesia, tingling or hyperkeratosis) without pain
2	Skin changes (e.g.,peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL
3	Severe skin changes (e.g., peeling, ulceration, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self-care ADL

ADL=activities of daily living
[a]HFSR or palmar-plantar erythrodysesthesia syndrome, a disorder characterized by redness, marked discomfort, swelling, and tingling in the palms of the hands or the soles of the feet;
[b]More specifics examples to grade 1 and grade 3 are added to facilitate proper grading [from the sorafenib package insert (West Haven, CT: Bayer Pharmaceuticals Corporation; 2007); Porta et al 2007].

Diarrhea should be graded according to CTCAE version 4.03 as described below in Table 8-5.

Table 8-5 CTCAE grading of diarrhea

Grade	Description
1	Increase of < 4 stools per day over baseline; mild increase in ostomy output compared to baseline
2	Increase of 4-6 stools per day over baseline; moderate increase in ostomy output compared to baseline
1-2 Complicated	Definition as above with the following complicating signs/symptoms: - Moderate to severe cramping - Grade ≥ 2 nausea/vomiting - Decreased performance status - Fever - Sepsis - Neutropenia - Frank bleeding - Dehydration - Unresolved diarrhea after 48 hours of treatment with loperamide (including high dose administration) and initiation of second-line treatment
3	Increase of ≥ 7 stools per day over baseline; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting selfcare ADL
4	Life threatening consequences; urgent intervention indicated

ADL=activities of daily living

Weight Gain

As weight gain can be considered a desired effect in children who are growing, weight gain will be assessed as follows:

- For any weight gain ≥ 20% above the baseline growth curve, evaluation of underlying organ function should be completed (including, but not limited to, echocardiogram, thyroid function studies and renal function panel).
- If underlying organ dysfunction is found and/or if weight gain is associated with generalized edema, patient discomfort or concern that the weight gain may have negative health consequences, the weight gain should be graded as an AE as per the CTCAE guidelines.

- If the patient is asymptomatic and no underlying organ dysfunction is found as detailed above, the primary study team may consider the weight gain to be a desirable effect. In this case, weight gain would not be considered an adverse event and therefore would not be graded as such.

8.2 Binimetinib dose modification guidelines

Pediatric Subjects

For pediatric patients who do not tolerate the 32 mg/m^2 BID dosing schedule (per tables 8.6 and 8.7), dose adjustment to 24 mg/m^2 is permitted in order to allow the patient to continue on study drug, but dose level -1 may not exceed 30 mg/dose for any subject. Further dose adjustment to 16 mg/m^2 and 12 mg/m^2 will be permitted following the same rules as outlined in the dose modification tables 8.6 and 8.7, and as per Appendix XIX and XX. Changes must be recorded on the specific section of the CRF.

A dose reduction below 12 mg/m^2 is not allowed. Patients with AEs that require a dose modification at the 12 mg/m^2 BID dose must be discontinued from study treatment. Dose interruptions of more than 28 days are not allowed.

Pediatric Stratum Dose Level	***Binimetinib* Dose**
0	*32 mg/m^2 BID*
-1	*24 mg/m2 BID*
-2	*16 mg/m2 BID*
-3	*12mg/m2 BID*

Adult Subjects

Initially, adult subjects (Stratum A) received binimetinib by mouth on a BID dosing schedule of 45mg/dose (the established adult RP2D). Because 4 of the first 11 enrolled subjects chose to remove themselves from study for "intolerable" skin AE's at the 45 mg BID dosing, the starting dose for the adult stratum is reduced from 45 mg BID to 30 mg BID in Protocol Version 2.0. Subjects who enrolled prior to protocol version 2.0 and tolerate binimetinib at 45 mg PO BID can continue on that dosing regimen if the treating physician believes that this is in the best interest of the patient. For adult patients who do not tolerate the 45 mg BID dosing schedule (per tables 8.6 and 8.7), dose adjustment to 30 mg BID is permitted in order to allow the patient to continue on study drug. For patients taking 30 mg bid further dose adjustment to 15 mg BID will be permitted following the same rules as outlined in the dose modification tables 8.6 and 8.7. Subjects who enrolled with protocol version 2.0 or later start binimetinib at 30 mg PO BID. For adult patients who do not tolerate the 30 mg BID dosing schedule (per tables 8.6 and 8.7), dose adjustment to 15 mg BID is permitted.

A dose reduction below 15 mg BID is not allowed. Dose interruptions of more than 28 days are not allowed. Changes must be recorded on the specific section of the CRF.

Adult Stratum Dose Level	***Binimetinib* Dose**
-1	*30 mg BID*
-2	*15 mg BID*

For all subjects

When the toxicity that resulted in a dose reduction improves to and remains stable at Grade 1 or less for a minimum of 28 days, the dose can be re-escalated at the investigators discretion provided there are no other concomitant toxicities. However, if the same toxicity occurs, the dose must be de-escalated again and re-escalation for a 2nd time is not permitted. Of note, **NO subjects were dose reduced to 30 mg PO BID or less, may not re-escalate their dose higher than 30 mg BID**.

No dose re-escalation is allowed after dose reduction due to left ventricular dysfunction or prolonged QTcF > 500 msec.

Dose reduction/interruption/discontinuation decisions should be based on the CTCAE grade of the toxicity and the guidelines provided below. In general, doses should not be reduced or interrupted for Grade 1 toxicities, but treatment to control symptoms should be provided as appropriate. All AEs should be followed weekly or as clinically appropriate until stabilization or resolution.

Please refer to section 8.3 for dose adjustment recommendations for binimetinib induced toxicities.

Table 8-6 Criteria for holding study drug

For details on management of specific adverse events, please see table 8.7. Please email study chairs **sabine.mueller@ucsf.edu**, **FisherM@email.chop.edu** and **Alyssa.reddy@ucsf.edu** if there is an AE that prompts a study drug hold.

TOXICITY	STUDY DRUG HOLD CRITERIA
Hematology	CTCAE grade 4 neutropenia lasting more than 7 consecutive days
	CTCAE grade 4 thrombocytopenia
	CTCAE Grade 3 or 4 neutropenia with fever (temperature ≥ 38.5°C)
Skin and subcutaneous tissue disorders	Rash, HFSR or photosensitivity CTCAE Grade 3 for> 7 consecutive days despite maximum skin toxicity treatment (as per local practice)
	Rash, HFSR or photosensitivity CTCAE Grade 4
Eye disorders	CSR (Retinopathy) CTCAE Grade 2 for > 14 consecutive days confirmed by ophthalmologic examination
	CSR (Retinopathy) or other Visual event CTCAE Grade ≥ 3, confirmed by ophthalmologic examination
Gastro-intestinal	≥ CTCAE grade 3 nausea or vomiting ≥ 48 hrs despite optimal anti-emetic therapy
	≥ CTCAE grade 3 diarrhea ≥ 48 hrs despite optimal anti-diarrhea treatment
Hepato-biliary	≥ CTCAE grade 3 total bilirubin
	≥ CTCAE grade 3 ALT (isolated increases in AST without concomitant increases in ALT will not be considered dose-limiting, because of the non-specific nature of AST)
	Serum alkaline phosphatase CTCAE Grade 4 for > 7 consecutive days
ECG QT Interval	QTc interval ≥ 501 ms confirmed on a least 2 ECGs
Renal	Serum creatinine > 2x ULN
Non-hematologic events	≥ CTCAE grade 3, except for the exclusions noted below
The following do not require holding drug	≥ CTCAE Grade 3 alopecia

8.3 Criteria for dose-modification in case of suspected binimetinib toxicity and re-initiation of treatment

Table 8-7 Binimetinib – Recommended dose modifications associated with treatment-related adverse events

Worst toxicity CTCAE v4.03 Grade (unless otherwise specified[a])	**Dose adjustment for binimetinib**
Eye disorder - CSR like events	
Grade 1	Maintain dose level of binimetinib and increase frequency of ophthalmic monitoring to at least every 14 days
Grade 2 or 3	Interrupt binimetinib and refer the patient to ophthalmologist within one week: - If resolved to Grade ≤ 1 in ≤ 28 days, reduce 1 dose level[b] of binimetinib - If not resolved to Grade ≤ 1 in ≤ 28 days, discontinue patient from study drug treatment
Grade 4	Permanently discontinue binimetinib and immediately follow up with ophthalmic monitoring[c]
Eye disorder - RVO	
RVO of any grade	Permanently discontinue binimetinib
Other eye disorders (i.e. retinal detachment)	
Grade 1	Maintain dose level of binimetinib and increase frequency of ophthalmic monitoring to at least every 14 days
Grade 2 - 3	Interrupt binimetinib and refer patient to ophthalmologist within one week: - If resolved to Grade ≤ 1 in ≤ 28 days, reduce 1 dose level[b] of binimetinib - If not resolved to Grade ≤ 1 in ≤ 28 days, permanently discontinue binimetinib
Grade 4	Permanently discontinue binimetinib
Liver related adverse events	
AST or ALT Grade 1 (> ULN - 3 x ULN)	Maintain dose level of binimetinib
Grade 2 AST or ALT (> 3 - 5.0 x ULN or 3 x baseline value) AND blood bilirubin* ≤ 2.0 x ULN	Interrupt dose of binimetinib until resolved to Grade ≤1, then: - If resolved in ≤ 14 days, maintain dose level of binimetinib - If resolved in > 14 days, reduce dose level of binimetinib
AST or ALT (> 3.0 - 5.0 x ULN) AND blood bilirubin* > 2.0 x ULN	Interrupt dose of binimetinib until resolved to Grade ≤ 1, then: - If resolved in ≤ 7 days, reduce dose level of binimetinib. - If resolved in > 7 days, discontinue patient from study drug treatment.
Grade 3 AST or ALT (> 5.0 - 8.0 x ULN) AND blood bilirubin* ≤ 2.0 x ULN	Interrupt dose of binimetinib until resolved to Grade ≤ 1), then: - If not resolved to Grade ≤ 1 in ≤ 28 days, permanently discontinue binimetinib - If resolved to Grade ≤ 1 in ≤ 28 days, reduce 1 dose level of binimetinib
AST or ALT (>8 x ULN) AND blood bilirubin* ≤ 2.0 x ULN	Permanently discontinue binimetinib
AST or ALT (> 5.0 x ULN) AND blood bilirubin* > 2.0 x ULN	Permanently discontinue binimetinib

Worst toxicity CTCAE v4.03 Grade (unless otherwise specified[a])	**Dose adjustment for binimetinib**
AST or ALT Grade 4 (> 20.0 x ULN)	First occurrence Grade 4: Withhold binimetinib for up to 4 weeks. - If improved to Grade 0 or 1, or Grade ≤2 if liver metastasis, then resume at reduced dose. - If not improved to Grade 0 or 1 within 4 weeks, permanently discontinue. Recurrent Grade 4: Permanently discontinue binimetinib
Cardiac disorders (dose adjustment for binimetinib only)	
Left ventricular systolic dysfunction[a] Asymptomatic decrease of >10% in LVEF compared to baseline and the LVEF is below the institution's lower limit of normal	Interrupt dose of binimetinib and repeat evaluation of LVEF within 2 weeks If the LVEF recovers (defined as ≥ LLN and decrease ≤ 10% compared to baseline) ≤ 4 weeks, reduce 1 dose level[b]. Monitor LVEF 2 weeks after restarting on binimetinib, every 4 weeks for 12 weeks and subsequently as per protocol. If the LVEF does not recover within 4 weeks, permanently discontinue patient from study treatment. Closely monitor LVEF until resolution (or 16 weeks).
Grade 3 – 4	Permanently discontinue patient from binimetinib. Closely monitor LVEF until resolution (or 16 weeks) Note: Copies of ECHO and/or MUGA scans could be requested for patients with decrease of >10% in LVEF compared to baseline and LVEF < LLN
CK elevation (dose adjustment for binimetinib only)	
Grade 1-2	Continue treatment on same dose level (If total CK ≥ 3 X ULN, measure isoenzymes and myoglobin in blood and urine
Grade 3 (> 5.0 - 10.0 x ULN)	If asymptomatic, maintain dose of binimetinib. Monitor closely and measure isoenzymes and myoglobin in blood and urine If symptomatic (muscle pain/spasms), interrupt dose of binimetinib until resolved to CTCAE Grade ≤ 1 and monitor closely then: - If resolved in ≤ 28 days, reduce 1 dose level[b] of binimetinib - If resolved in > 28 days, permanently discontinue binimetinib
Grade 4	If asymptomatic, interrupt dose of binimetinib and monitor closely - If resolved in ≤ 28 days, reduce 1 dose level[b] of binimetinib - If resolved in > 28 days, permanently discontinue binimetinib If symptomatic, permanently discontinue binimetinib
QTc Prolongation	
QTcF > 500 msec (Grade 3) (confirmed by at least 2 ECGs 5 minutes apart)	1st occurrence: a repeat ECG confirming the prolonged QTc should be obtained. While waiting to have the 2nd ECG completed, treatment with binimetinib should be temporarily suspended. Electrolyte abnormalities, if any, should be corrected and any concomitant medication that may potentially prolong QT should be discontinued prior to repeating the ECG. Patient monitoring until resolution of the adverse event should be implemented in the hospital including a consultation with a cardiologist. Once the QTc prolongation has resolved (CTC Grade < 1), binimetinib may be restarted at a reduced dose. 2nd occurrence: If a second episode occurs that is attributed to binimetinib, study drug must be permanently discontinued.
Rash	
Grade 1	Treatment with binimetinib should be maintained at the current dose *Initiate prophylactic regimen if it was not already started and monitor closely*

Worst toxicity CTCAE v4.03 Grade (unless otherwise specified[a])	**Dose adjustment for binimetinib**
Grade 2	1st occurrence: Treatment with binimetinib should be maintained at the current dose and rash should be closely monitored. *Initiate prophylactic regimen if it was not already started.* Reassess within a maximum of two weeks. If rash worsens or does not improve, current dosing may be continued, as long as it is not intolerable to patient. and does not increase in grade. If rash worsens or does not improve, and is intolerable to patient, interrupt dosing until improvement to Grade ≤ 1. Resume treatment at reduced dose level. If there is no re-occurrence of grade 2 rash, the treating physician has the option to re-escalate after 28 days if thought to be in the best interest of the patient. 2nd occurrence: Treatment with binimetinib should be dose reduced and rash should be closely monitored. Reassess within a maximum of two weeks. If rash worsens or does not improve, interrupt dosing until improvement to Grade ≤ 1. Resume treatment at reduced dose level. If there is no re-occurrence of grade 2 rash, the treating physician has the option to re-escalate after 28 days if thought to be in the best interest of the patient.
Grade 3	1st occurrence: Treatment with binimetinib should be interrupted. Reassess the patient weekly. *Consider referral to dermatologist and manage rash per dermatologist's recommendation*. -Interrupt treatment until improvement to Grade ≤ 1. Resume treatment with binimetinib at reduced dose level. If there is no re-occurrence of grade 3 rash, the treating physician has the option to re-escalate after 4 weeks if though to be in the best interest of the patient. 2nd occurrence: Interrupt treatment until improvement to Grade ≤ 1. Resume treatment with binimetinib at a reduced dose level. *Consider referral to dermatologist and manage rash per dermatologist's recommendation*
Grade 4	Permanently discontinue binimetinib
Diarrhea	
Grade 1-2	Consider temporary interruption of binimetinib until resolved to Grade ≤ 1. Treatment with binimetinib may then be resumed at the same dose level
Grade 3	Temporarily interrupt binimetinib treatment until resolved to Grade ≤ 1. If diarrhea resolves within 3 days to grade 1 or less, patient can be restarted on the same dose level. If > 3 days, restart binimetinib at a reduced dose level
Grade 4	Temporarily interrupt binimetinib treatment until resolved to Grade ≤ 1. Restart binimetinib at a reduced dose level
Nausea/Vomiting	
Grade 1-2	Treatment with binimetinib should be maintained at the current dose. Promptly institute antiemetic measure.
Grade 3	Temporarily interrupt binimetinib treatment until resolved to Grade ≤ 1. Resume treatment with binimetinib at the same dose if, in the judgment of the investigator, the toxicity is considered to be unrelated to binimetinib, or at one reduced dose level. Note: Interupt dose for ≥ grade 3 vomiting or grade 3 nausea only if the vomiting or nausea cannot be controlled with optimal antiemetics (as per local practice)
Grade 4	Permanently discontinue binimetinib treatment
All other adverse events (suspected to be related)	
Grade 2 (intolerable) adverse reactions	- Withhold binimetinib for up to 4weeks or until resolved to Grade 0 or 1 and restart at a reduced dose. If there is no re-occurrence of the grade 2 intolerable adverse event, the treating physician has the option to re-escalate after 4 weeks if thought to be in the best interest of the patient.
Grade 3 or 4	Withhold binimetinib for up to 3 weeks. - If improved to Grade 0 or 1 or pretreatment/baseline level, resume at reduced dose. - If not improved, permanently discontinue.

[a] Not according to CTCAE

Worst toxicity CTCAE v4.03 Grade (unless otherwise specified[a])	Dose adjustment for binimetinib

[b] Dose reduction below 15 mg (for adults) or 12 mg/m^2 (for children) BID for binimetinib is not allowed
[c] Ophthalmic monitoring recommended: further evaluation with specialized retinal imaging (e.g. ocular coherence tomography, angiography)
***Refers to total bilirubin**

Weight Gain
For patients who experience ≥ grade 3 weight gain, binimetinib can be restarted when weight has resolved to ≤ grade 2 at investigator discretion.

8.4 Toxicities Requiring Removal from Protocol Therapy

For the adult stratum: Any toxicity defined in section 8.2 or 8.3 that is at least possibly related to study medication that recurs on the lowest dose of binimetinib (dose level -2). No subject may be dose-reduced past dose level -2.

For the pediatric stratum: Any toxicity defined in section 8.2 or 8.3 that is at least possibly related to study medication that recurs on the lowest dose of binimetinib (dose level -3). No subject may be dose-reduced past dose level -3.

Any ≥ Grade 3 toxicity that is at least possibly due to study drug that does not resolve to ≤ Grade 1 within the guidelines in section 8.3 requires dose reduction by one dose level. If the subject cannot be further dose reduced, then this requires removal from protocol therapy.

Development of retinal vein occlusion of any grade will also require removal from protocol therapy.

Development of glaucoma, intraocular pressure >21 mmHg, or any other significant abnormality on ophthalmic examination (performed by an ophthalmologist) while on study treatment will require removal from protocol therapy. Ophthalmological findings secondary to long-standing Optic Pathway Glioma such as optic nerve pallor or strabismus will NOT be considered significant for the purposes of the study.

The study chair, the study co-chair and the NF Operations Center should be contacted for any questions regarding toxicity.

9.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Study evaluations are summarized in the observation table in Appendix I.

9.1 Required Clinical, Laboratory and Disease Evaluation

All entry/eligibility studies must be performed within 2 weeks prior to enrollment into the trial (unless otherwise specified). The baseline MRI study documenting disease status is required within 4 weeks prior to enrollment. Entry/eligibility studies (laboratory and MRI) may be counted as the baseline (pre-study) studies as long as there has been no concerning change in clinical status prior to starting study drug. Study drug must start within 14 days of enrollment.

All studies to determine eligibility for Re-Treatment must be performed within 2 weeks prior to "enrollment" on the Re-Treatment arm except for MRI, ophthalmology, Echo, and EKG (which is required within 4 weeks prior to "enrollment" on the Re-Treatment Arm), unless otherwise indicated. Re-Treatment eligibility studies may be counted as the Re-Treatment baseline studies as long as there has been no concerning change in clinical status prior to starting study drug, except for pregnancy testing in females of childbearing age which must be negative within 7 days prior to re-starting binimetinib. Study drug must start within 14 days of "enrollment" on the Re-Treatment Arm.

9.2 History and physical examination and vital signs

Initial Treatment
Physical examination will be performed prior to entry, at the end of courses 1 (±1week); 2 (±1week), 3 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. The exam must comprise a total body examination (general appearance, skin, neck, including eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and basic nervous system). This should also include a neurological exam. Concomitant medications should be reviewed at each visit. Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded.

Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature, and weight.

Complete medical history should be recorded at the baseline visit.

Re-Treatment
Physical examination will be performed prior to entry, at the end of courses 2 (±1week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), physical examination should be performed after every 3 cycles (27, 30, 33, 36) ±2 weeks.

The exam must comprise a total body examination (general appearance, skin, neck, including eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and basic nervous system). This should also include a neurological exam. Concomitant medications should be reviewed at each visit. Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded.

Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature, and weight.

Complete medical history should be recorded at the baseline visit.

9.3 Ophthalmological Toxicity Exam

Initial Treatment
Subjects will have a standard ophthalmological exam with emphasis on corneal, lens and retinal changes. In subjects with orbital plexiform neurofibromas the intraocular pressure should be measured at baseline and subsequently if feasible. Exams should occur prior to entry, and post courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment
All subjects will have a standard ophthalmological exam with emphasis on corneal, lens and retinal changes. In subjects with orbital plexiform neurofibromas the intraocular pressure should also be measured at baseline and subsequently if feasible. Exams should occur prior to entry, and post courses 6 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), ophthalmological exam should be performed after every 6 cycles (30, 36) ±2 weeks.

Subject with evidence of disease (retinal vein occlusion) should have a full evaluation (such as fluorescein angiography and/or optical coherence tomography) as per institutional guidelines.

For details how to record visual functioning please refer to Appendix XII.

9.4 Hematology

Initial Treatment
CBC must include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. CBC must be performed within two weeks prior to entry on trial, at the end of courses 1 (±1 week), 2 (±1 week), 3 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment
CBC must include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. CBC must be performed within two weeks prior to entry, at the end of courses 2 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), hematology labs should be performed after every 3 cycles (27, 30, 33, 36) ±2 weeks.

9.5 Blood chemistry

Initial Treatment
Blood chemistry must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, and CPK. Blood chemistries should be drawn within two weeks prior to study entry, at the end of courses 1 (±1 week), 2 (±1week), 3 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment
Blood chemistry must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, and CPK. Blood chemistries should be drawn within two weeks prior to entry, at the end of courses 2 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements

are being made to transition the participant to a MEK inhibitor off study (see section 6.2), chemistry labs should be performed after every 3 cycles (27, 30, 33, 36) ±2 weeks.

9.6 Serum or Urine Pregnancy Test

Initial Treatment
Standard pregnancy tests will be given to all females of childbearing age within 7 days prior to starting treatment medication. In addition, tests will be administered post courses 1 (±1 week), 2 (±1week), 3 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks) to ensure that females are not pregnant while on the study medication. In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment
Standard pregnancy tests will be given to all females of childbearing age within 7 days prior to starting treatment medication. In addition, tests will be administered at the end of courses 2 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), standard pregnancy testing should be performed after every 3 cycles (27, 30, 33, 36) ±2 weeks.

9.7 ECHO (or MUGA) and ECG

Initial Treatment
Standard ECHO (or MUGA) and ECG should be obtained within 4 weeks prior to study entry and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above.

Re-Treatment
Standard ECHO (or MUGA) and ECG should be obtained within 4 weeks prior to study entry and after courses 6 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), standard ECHO (or MUGA) and ECG should be performed after every 6 cycles (30, 36) ±2 weeks.

9.8 MRI for volumetric analysis of plexiform neurofibroma (Appendix IV)

Initial Treatment
Evaluate the plexiform neurofibromas(s) by MRI within 4 weeks prior to enrollment and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. To evaluate the durability of response, subjects who responded while on treatment (≥20% target lesion shrinkage by course 12), should have a follow-up MRI scan at approximately 4 months and 12 months following completion of therapy. Of note, this scan does not have to be performed at the study site but needs to be submitted for evaluation by the enrolling site. If a subject starts another tumor-directed therapy during the one year follow-up time, then they are off-study and no further MRIs requested.

Re-Treatment
Evaluate the plexiform neurofibromas(s) by MRI within 4 weeks prior to entry and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), MRI should be performed after every 6 cycles (30, 36) ±2 weeks.

The imaging protocol outlined in Appendix IV will be used each time MRI examinations are performed to assess progression or response. In subjects with clinical suspicion of disease progression, MRI analysis should be performed earlier using the protocol outlined in Appendix IV. If because of unforeseen reasons, an MRI time window is passed, but not yet at the time of the opening of the subsequent window, the MRI should be performed and the protocol violation noted.

9.9 Photographs and Functional Evaluations (Appendix XVI) (*Initial Treatment only*)

Subjects will take photographic documentation of visible target PN (Appendix XVI) and functional evaluations based on tumor location (Appendices XII-XIV) at the following time points: within 4 weeks prior to course 1 and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. Functional assessments are ideally carried out by the same person for any given subject.

9.10 Plexiform Neurofibroma Clinician Morbidity Assessment (Appendix XI)

Initial Treatment
This form should be completed by the treating physician at baseline and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. This assessment is ideally carried out by the same person for any given subject.

Re-Treatment
This form should be completed by the treating physician at baseline and after courses 4 (±2 weeks), 8 (±2 weeks), 12 (±2 weeks), 18 (±2 weeks), and 24 (±2 weeks) while on study and at end of study (±2 weeks). In addition, at the end of treatment (±2 weeks) if this is not encompassed in the times above. This assessment is ideally carried out by the same person for any given subject. This is not required for participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2).

9.11 Binimetinib diary (Appendix V)

Initial Treatment
A diary will be kept by the subject and/or proxy (see Appendix V). A document developed by the local institution can be used as a substitute for Appendix V. The diary will be reviewed with the treating physician at the history/physical visit at the end of courses 1 (±1week); 2 (±1week), 3 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment

A diary will be kept by the subject and/or proxy (see Appendix V). A document developed by the local institution can be used as a substitute for Appendix V. The diary will be reviewed with the treating physician at the history/physical visit at the end of courses 2 (±1 week), 4 (±1 week), 6 (±2 weeks), 8 (±2 weeks), 10 (±2 weeks), 12 (±2 weeks), 15 (±2 weeks), 18 (±2 weeks), 21(±2 weeks), and 24 (±2 weeks). In addition, at the end of treatment ±2 weeks if this is not encompassed in the times above. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), diary should be reviewed with the treating physician after every 3 cycles (27, 30, 33, 36) ±2 weeks.

9.12 Plasma Cytokines and Growth Factors (Optional) (Appendix VII) (*Initial Treatment only*)

Procedure: 10 ml blood will be drawn into EDTA tubes before treatment initiation, post course 1 and post course 4. The sample will be collected at least 30 min but no more than 2 hours after taking the morning dose. Any remaining specimens will be stored for future research with subject consent. See Appendix VII for more details.

Maximum blood volumes for cytokine studies:
Institutional guidelines will be followed for maximum blood volumes for research samples. The table below provides details regarding blood volumes obtained for research studies during the study.

Type of test	Volume per sample (ml)	Number of samples (volume for all samples, ml)
Plasma Cytokines and Growth Factors	10	3 (30)
Total volume for research samples		3 (30)

9.13 Biological Specimens for banking (Appendix XXI)

Initial Treatment (only)
Blood Samples will be drawn at baseline (pre-treatment), after course 4 (+2 weeks), after course 12 (+2 weeks), and at the end of treatment (+2 weeks) if this is not encompassed in the times above. In addition, blood samples should be drawn around the time of tumor biopsy if tumor tissue is being submitted.

The following will be collected at each time point:

- Streck tube (for plasma): ~ 2 mL.
- EDTA tube (for DNA): ~ 5 mL.
- PAXGene tube (for RNA) ~ 2.5 mL.

Tubes should be inverted at least 10 times after being drawn.

The samples will only be obtained if the total blood volume does not exceed 5 mL/kg. If not enough blood can be drawn for all tubes, the priority order for obtaining the specimens is:
1) Streck tube, 2) EDTA tube, 3) PAXGene tube.

Label the specimens with the specimen labels provided with the specimen kit.

Blood specimens must be shipped (unprocessed) same day at ambient temperature to the biorepository by overnight delivery using shipping container provided by the biorepository (see Appendix XXI).

Any time on study (Initial Treatment or Re-Treatment)
Tumor Tissue (optional) will be collected from subjects undergoing a clinically indicated procedure and who consent:

- Fresh frozen tissue: 1-2 pea sized aliquots (~ 0.3 cm3, equivalent to 0.8 cm in diameter) of excess (left over) tumor tissue. Place each aliquot in a separate cryovial and label the specimen with subject's study number. Snap freeze the aliquots in liquid nitrogen (preferred) or dry ice and then store in a freezer at -80°C or below until they can be sent to the biorepository.
- 1 H&E-stained slide cut from each submitted piece to confirm tumor is present.
- If fresh frozen tissue is unavailable, please submit processed DNA (at least 3 micrograms) and RNA (at least 400 nanograms with optimal RIN > 7) if available.

Fresh frozen tissue or DNA/RNA must be shipped on dry ice via overnight delivery using shipping materials provided by the biorepository (see Appendix XXI). H&E slide should be shipped at ambient temperature.

9.14 Baseline PN location and morbidity form (Appendix X) (*Initial Treatment only*)

This form should be completed by the Site PI at the time of enrollment.

9.15 Symptom checklist completed by subject or their guardian (Appendix IX)

Initial Treatment
This form should be completed pre-treatment, after course 4 ($\pm$2 weeks), 8 ($\pm$2 weeks), 12 ($\pm$2 weeks), 18 ($\pm$2 weeks), and 24 ($\pm$2 weeks). In addition, at the end of treatment ($\pm$2 weeks) if this is not encompassed in the times above.

Re-Treatment
This form should be completed pre-treatment, after course 4 ($\pm$2 weeks), 8 ($\pm$2 weeks), 12 ($\pm$2 weeks), 18 ($\pm$2 weeks), and 24 ($\pm$2 weeks). In addition, at the end of treatment ($\pm$2 weeks) if this is not encompassed in the times above. This is not required for participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2).

9.16 Patient-reported Outcomes (PROs): Quality of Life (QOL) and Pain Evaluations (Required)

This study will evaluate quality of life, pain intensity, and pain interference in subjects during treatment with binimetinib. Age-appropriate patient-reported outcome (PRO) measures will be administered based on a Pediatric Module and an Adult Module.

The Pediatric Module (ages 1- 17 at study entry) will consist of the self-report and parent proxy Pediatric Quality of Life Inventory (PedsQL) Generic Module (Toddler, Child, Teen, and Young Adult forms) to assess QOL. Pain intensity will be assessed by self-report with the Numeric Rating Scale-11 (NRS-11) and pain interference will be measured by the Pain Interference Index (PII).

The Adult Module (ages $\geq$ 18 years at study entry) will consist of the Adult PedsQL™ NF1 Module (Adult self-report form) and the PlexiQoL to assess disease-specific QOL. Pain intensity will be assessed by self-report with the Numeric Rating Scale-11 (NRS-11) and pain interference will be measured by the Brief Pain Inventory (BPI) Pain Interference Scale.

The following table specifies the PRO module (pediatric or adult) to be administered by age of the subjects at study entry. **The specific PRO measures to be administered within each module are specified in Appendix VIII.**

PRO Module Administered by Age of Subject at Study Entry*

Module of PRO questionnaires	Age of Subject (years) at **Study Entry**	
	1-17	≥18
Pediatric PRO Module*	X	
Adult PRO Module*		X

***See Appendix VIII for specific PRO questionnaires to administer per module, a detailed description of each measure, and important administration instructions.**

PRO Assessment Schedule

Initial Treatment
Baseline and follow-up PRO assessments
The PRO forms should be completed pre-treatment, and again after course 4 ±2 weeks, 8 ±2 weeks, 12 ±2 weeks, 18 ±2 weeks, and 24 ±2 weeks. In addition, they should be administered at the end of treatment ±2 weeks if this is not encompassed in the times above.

Re-Treatment
Baseline and follow-up PRO assessments
The PRO forms should be completed pre-treatment, and again after course 4 ±2 weeks, 8 ±2 weeks, 12 ±2 weeks, 18 ±2 weeks, and 24 ±2 weeks. In addition, they should be administered at the end of treatment ±2 weeks if this is not encompassed in the times above. This is not required for participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2).

Transmission of Completed Forms
Completed and checked PRO forms should be sent (email copies) to the NF Consortium Operations Center within 2 weeks of completion along with the NF QOL Cover sheet to record the forms being sent and explain any issues with administration. Please make notes about any unclear responses or problems encountered. For any questions regarding the QOL/pain assessment please contact: Karin Walsh at KWalsh@childrensnational.org.

PRO Central Review
The QOL documents will be reviewed centrally by Dr. Karin Walsh. If any responses are noted that suggest serious emotional distress, the PI at the subject's site will be notified but please note that central review will be conducted in real time. The site PI will be responsible for acknowledging to Dr. Walsh receipt of these concerns, and for initiating appropriate action as deemed clinically necessary.

9.17 Phone Call (*Re-Treatment arm only*)
End of course 1 (±1week). To assess drug compliance and toxicity.

10.0 ADVERSE REPORTING REQUIREMENTS

10.1 Definitions

- **Adverse Events**: An adverse event is any new, undesirable medical occurrence or change (worsening) of an existing condition in a subject that occurs during treatment, whether or not considered to be product related. Therefore, adverse events are treatment emergent signs or symptoms. Elective hospitalizations for pretreatment conditions (e.g., elective cosmetic procedures) are not adverse events. Non-clinically significant abnormal laboratory values should not be reported as adverse events; however, any clinical consequences of the abnormality should be reported as adverse events. All adverse events must be noted on the case report forms and submitted to the operations center within 2 weeks of completion of every treatment course.

  For all adverse events, the site investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification (within 24 hours) to the Protocol PI and NF Consortium Operations Center clinical research nurse manager. Follow-up of the adverse event, even after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and sponsor.
- **Serious Adverse Events**: A serious adverse event is defined by regulatory agencies as one that suggests a significant hazard or side effect, regardless of the investigator's or sponsor's opinion on the relationship to investigational product.

  This includes, but may not be limited to, any event that (at any dose):
  - is FATAL
  - is LIFE THREATENING (places the subject at immediate risk of death);
  - requires HOSPITALIZATION or prolongation of existing hospitalization;
  - is a persistent or significant DISABILITY/INCAPACITY; or
  - is a CONGENITAL ANOMALY/BIRTH DEFECT

Important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious. Examples include allergic bronchospasm, convulsions, and blood dyscrasias. A planned medical or surgical procedure is not, in itself, an SAE.

10.2 Common Terminology Criteria for Adverse Events (CTCAE) V.4.03

This study will utilize the CTCAE of the National Cancer Institute for reporting and grading of adverse events. A copy of the current version of the CTCAE version 4.03 can be downloaded from the CTEP home page: http://ctep.cancer.gov/reporting/ctc.html.

10.3 Attribution: Definitions of relationship to study medication are as follows:

- UNRELATED: bears no relation to timing of medication, similar to symptoms or signs expected in the disease process, does not recur on re-challenge.

- UNLIKELY: does not have temporal relationship to intervention, could readily have been produced by the subject's clinical state, environmental, or other interventions, does not reappear or worsen with reintroduction of intervention.
- POSSIBLY: bears relation to timing of medication, similar to symptoms or signs expected in the disease process, does not recur on re-challenge.
- PROBABLY: bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, does not recur on re-challenge.
- DEFINITELY: bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, occurs on re-challenge.

The expected adverse events related to administration of binimetinib are listed in Section 6.1.4. All other adverse events not attributed to study drug will be considered unexpected. Adverse events attributable to study drug will be reported if the adverse events are at an intensity that is more severe than previously documented or considered significant by the investigator.

10.4 Reporting Procedures for All Adverse Events

All observed or volunteered adverse events, regardless of suspected causal relationship to study drug, will be recorded as Adverse Events in the case report forms and submitted to the Operations Center within 2 weeks of completion of each treatment course. Events involving adverse drug reactions, illnesses with onset during the study, or exacerbation of pre-existing illnesses should be recorded. Objective test findings (e.g., abnormal laboratory test results) should also be recorded.

It will be left to the investigator's clinical judgment whether or not an adverse event is of sufficient severity to require that the subject should be removed from treatment. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse event. If either of these occurs, the subject will be given appropriate care under medical supervision until symptoms cease or until the condition becomes stable.

Adverse events will be assessed by the treating investigator for relationship to the study drug (unrelated, unlikely, possible, probable, or definitely related).

10.5 Expedited Reporting Guidelines

The following adverse events require expedited reporting:

- All adverse events that are both serious and unexpected per section 6.1.4
- Adverse events that, in the opinion of the treating physician, might influence the benefit-risk assessment of administration of binimetinib as outlined in the protocol
- All grade 5 adverse events
- A serious adverse event that occurs within 30 days of the last dose of the investigational agent
- In addition, if a male who has been exposed to binimetinib within 28 days prior to the time of conception, impregnates a female partner, then the female partner and fetus are considered "exposed during pregnancy", and this event is reportable as an SAE irrespective of the presence of an associate AE/SAE.

- In the case of an exposed female and fetus, follow-up will be conducted to obtain general information on the pregnancy (both the female and the fetus) and its outcome until the infant is 1 month postnatal. Neonatal deaths that occur within one month of birth should be reported, irrespective of causality, as SAEs. In addition, infant deaths after one month should always be reported when the investigator assesses the infant death as related to exposure to the investigational product.

- Expedited AE reporting timelines defined:
"24 hours; 24 hours" – The investigator must initially report the AE within 24 hours (or immediately if the event is fatal or life-threatening) of learning of the event to the Study Chairs (sabine.mueller@ucsf.edu), FisherM@email.chop.edu, and Alyssa.reddy@ucsf.edu, and NF Operations Center (nfcop@uab.edu) followed by a complete written report within 24 hours of the initial 24-hour report.

Adverse events requiring expedited reporting will be reported and documented on **Form FDA 3500A MedWatch Form** http://www.fda.gov/medwatch/getforms.htm) and forwarded to:

NF Consortium Operations Center	
Clinical Research Manager: Lynn Merritt, RN	205-934-5376
Program Director: Karen Cole – Plourde, BS	205-934-5140
Research Compliance Manager: Juliette Southworth, BS	205-975-4075
Direct Email: nfcop@uab.edu	

The clinical research nurse manager from the NF Consortium Operations Center will forward all related adverse events that are both serious and unexpected to Dr. Mueller and the PNOC operation's office on **Form FDA 3500A MedWatch Form** http://www.fda.gov/medwatch/getforms.htm for submission to the FDA.

MedWatch
Fax: 1-800-FDA-0178

Research Monitor, DSMB, NF Consortium IRB and other designated regulatory agencies will also receive MedWatch Form documents for adverse events requiring expedited reporting.

10.5.1 Array BioPharma, a wholly owned subsidiary of Pfizer Inc. Expedited Reporting Guidelines

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided main informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Array within 24 hours of learning of its occurrence.

Any SAEs experienced after this 30 day period should only be reported to Array if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and send the

completed, signed form by fax within 24 hours to the oncology Array Drug Safety department.

PPD Pharmacovigilance (PVG) Fax: 1-888-488-9697

The original copy of the SAE Report Form and the fax confirmation sheet, provided by NF Consortium Operations Center, must be kept with the case report form documentation at the site.

Follow-up information is sent to the same contact(s) to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure and is thought to be related to the Array study treatment, an oncology Array Drug Safety associate may urgently require further information from the investigator for Health Authority reporting. Array may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

10.6 Reporting of Protocol Violations/Deviations and Unanticipated Problems

Site reporting to NF Operations Center
Sites will report unanticipated problems and/or protocol deviations that impact subject safety or the scientific integrity of the study to the Operations Center promptly at nfcop@uab.edu. Other protocol violations and deviations should be reported to the NF Operations Center annually.

NF Operations Center reporting requirements
The Operations Center will report unanticipated problems that impact subject safety or the scientific integrity of the study to the USAMRMC ORP HRPO promptly. Unanticipated problems will also be reported to the protocol team, Research Monitor, FDA, NF Consortium IRB, Array BioPharma, a wholly owned subsidiary of Pfizer Inc., and DSMB.

All unanticipated problems involving risk to subjects or others must be promptly reported by the NF Operations Center via phone (301-619-2165), email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

Other protocol violations and deviations that do not impact subject safety or affect scientific integrity of the study will be provided annually to the Sponsor.

11.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

11.1 Criteria for Removal from Protocol Therapy

a. Progressive disease (See Section 13) *compared with* pre-Treatment baseline for initial therapy.
b. Completion of course 24 of initial therapy
c. <20% reduction in target tumor volume (from baseline) after course 12 of initial therapy (Of note, subjects may begin course 13 while central review of tumor response is ongoing). *Of note, this does not apply to subjects on the Re-Treatment arm*.
d. Progressive disease (See Section 13) *compared with* pre-Re-Treatment baseline for Re-Treatment.
e. Completion of course 24 of Re-Treatment (unless "f" below applies).
f. For participants deriving benefit and for whom the treating team is working on transitioning the participant to a MEK inhibitor off study (see section 6.2), completion of course 36 of Re-Treatment or when off study MEK inhibitor is started, whichever is sooner.
g. Treatment delays ≥ 28 days
h. Refusal of further protocol therapy by subject/parent/guardian
i. Non-compliance that in the opinion of the investigator does not allow for ongoing participation.
j. Physician determines it is not in the subject's best interest.
k. Subject becomes pregnant
l. Subject is prescribed a non-allowed concomitant medicine during study.
m. Withdrawal of protocol consent
n. Any surgical procedure on a target PN.
o. Binimetinib-related toxicity requiring removal (see Section 8).

Subjects who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below).

11.2 Off Study Criteria

For subjects who did not have a response (<20% shrinkage of target tumor by course 12) or have tumor progression (≥ 20%) at any time:

a) Thirty days after the last dose of binimetinib (but note that subjects with ongoing toxicity should be followed until the toxicity resolves or 30 days, whichever is longer)

For subjects who had a response (≥20% target tumor shrinkage by course 12) and never experience tumor progression on initial treatment:

b) Completion of their one year follow-up MRI scan *unless they meet the criteria for "enrollment" on the Re-Treatment Arm*

For subjects who "enroll" on the Re-Treatment Arm and complete 24 courses:

c) Thirty days after the last dose of binimetinib (but note that subjects with ongoing toxicity should be followed until the toxicity resolves or 30 days, whichever is longer). *Note: for participants deriving benefit and for whom the treating team is working on transitioning the participant to a MEK inhibitor off study (see section 6.2), when off study MEK inhibitor is started.*

For all subjects (supercedes "a" or "b"):

d) Death
e) Lost to follow-up
f) Withdrawal of consent for any further data submission.

g) Initiation of subsequent other medical treatment directed towards the plexiform neurofibroma
h) Initiation of medical treatment (e.g. chemotherapy, biologic therapy, radiation therapy) directed towards other NF1-related tumor such as an optic pathway glioma

12.0 STATISTICAL AND ETHICAL CONSIDERATIONS

12.1 Subject Accrual

Subjects of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section 4.0. To date, there is no information that suggests differences in drug metabolism or disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Efforts will be made to extend the accrual to a representative population, but in a phase II study with limited accrual, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand, and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, age, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

12.2 Statistics and Feasibility

The primary objective of this study is to determine if there is an adequate level of disease responsiveness to binimetinib in children and adults with NF1 and inoperable plexiform neurofibromas.

Sample Size

Enroll 25 evaluable subjects per stratum (pediatric and adult). Target: minimum of 17 evaluable subjects per cohort. To allow for up to 25% unevaluable subjects, a maximum of up to 25 subjects per cohort will be enrolled. We anticipate enrollment to be completed within 12 months with the primary outcome measure defined in 24 months.

The sample size for this trial is based on the primary factors: Safety and feasibility data needed: thus, safety based on risk versus benefit. For feasibility, we expect at least efficacy of a 25% response rate. We are only interested if binimetinib is positive and thus, assume a 1-sided test using a Type I error of 5%. We also want to achieve reasonable power within each of the two strata to enable its evaluation separately. We assume a null hypothesis success rate of 5% in each stratum versus a treatment with a 25% response rate. If all 20 patients are evaluable there is 80% power to detect differences between 25% and 5% in each stratum with 80% power. The power in the strata is 64% when the minimal sample size is realized. If both strata perform the same, the power to reject the null hypothesis that the efficacy rate is 5% versus 25% is 95% when the minimal sample size is available (2n=34).

12.3 Statistical Analysis Plan

The basic statistical analysis plan for this proof-of-concept study will describe the subject population by baseline characteristics: clinical, PD, functional assessments, Quality of Life and pain scores at baseline. Following complete accrual, study logistics will be summarized as per a Consort Diagram with reasons for withdrawal or unable to complete the study tabulated. Appropriate data analysis sets will be defined. The full-analysis set will include data from all subjects who receive ≥1 dose of therapy on this study; a

safety analysis set will comprise data from subjects in the full-analysis set with any treatment doses. Other data sets (responding, evaluable, and pharmacodynamic data sets) will be defined and will include data from subjects who have the necessary baseline and on-study measurements to provide interpretable results for specific parameters of interest.

Descriptive summaries will be prepared to show sample size, mean, standard deviation, 95% confidence intervals (CIs) on the mean, median, minimum, and maximum for continuous variables and counts, percentages, and 95% CIs on the percentage for categorical variables. This will be done for each cohort separately. For endpoints relating to tumor control, subject well-being, and biomarkers, analyses will be done based on the full-analysis, responding, evaluable, or pharmacodynamic data sets, as appropriate. Comparison against the hypothesized control rate will be done using exact tests for a binomial with the full cohort and then by strata. Time-to-event analyses will be performed with reference to the date of first treatment on this study. Analyses will focus on evaluation of outcomes and will be largely descriptive in nature as per the sample size justification section within cohort. Changes in tumor volumes, etc. will be summarized by medians, ranges, and the corresponding 95% CI. Continuous and categorical variables will also be summarized as appropriate.

Based on the safety analysis set, information regarding study treatment administration, drug dosing and course compliance, safety variables, will be described and summarized. Safety will be assessed via tabulations of AEs and SAEs as well as Diary recordings following treatment dose/course of therapy.

All analyses for outcome results will be based on evaluable subjects, as defined in section 12.4.

An early stopping rule will be invoked for both Strata separately to potentially prevent accrual of subjects onto the study in the event that binimetinib is associated with a higher than acceptable rate of dose-limiting toxicity (DLT) requiring removal from study (set at 10% or higher) during the first 2 courses. Toxicity will be continuously monitored. As per the 2nd table below, if at any time >2 of the first 10 subjects or 4 or more of the first 15 total subjects are removed for toxicity as per Section 8.3, then accrual will be stopped until the DSMB reviews safety and efficacy data for the study and recommends termination or despite the DLT (because of the benefit:risk assessment or other reasoning) recommends reopening recruitment. Boundaries for DSMB for consideration of terminating each cohort (both stratum A and B) would be the same.

Table 1: Stopping Boundaries requiring DSMB to consider terminating trial

Number of subjects	Trial will be stopped if the number of subjects with severe toxicity is greater than or equal to the number below
10	3 (equivalent to a p-value of 0.07 that the rate of severe toxicity is 10% or less)
15	4 (equivalent to a p-value of 0.055 that the rate of severe toxicity is 10% or less)

Using these rules and the binomial distribution, the chance of putting the trial on hold and potentially stopping early within the first 10 subjects:

Table 2

True probability of toxicity	Chance of stopping early

.10	7%
.20	32%
.25	47%
.30	62%
.35	74%
.40	83%

In addition, if at any time during the trial a third subject experiences a grade 2 or higher ophthalmologic toxicity, the DSMB will be asked to review the toxicity data to date with estimates of the upper limit of adverse event rate, evaluate the benefits versus risks, and recommend whether any changes to the protocol are required. This review will not automatically halt enrollment, unless the toxicities noted are dose-limiting and trigger the early stopping rule noted above.

For the comparison of volumetric measures, 60 consecutive patients enrolled on Neurofibromatosis Clinical Trials Consortium PN studies will be included (including NF108). Two groups of independent volume measurements (NCI-MEDx and MGH-3DQI) will be performed of the target lesions on up to 5 MRIs per subject (pre-study, baseline, pre-cycle 5, pre-cycle 9, pre-cycle 13). Interval change will be calculated to classify disease status as follows: progressive disease (PD) if volume increase is $\geq$ 20%, partial response (PR) if volume decrease is $\geq$ 20%, and stable disease (SD) if change is < 20% between time points. Of note, the results of the MEDx analysis at the NCI will be utilized in analyzing and making decisions on the clinical trial, as has been done on all previous Neurofibromatosis Clinical Trials Consortium PN trials. The results of the 3DQI analysis of each scan performed at the MGH will not be released to sites or the study team while the study is ongoing. Wilcoxon signed rank test will be used to statistically compare the numerical difference between PN volumes at each time-point, and the numerical difference between the percent changes in PN volumes over time. The Jonckheere-Terpstra trend test will be used to establish the degree of significance of the association in disease status classifications. After establishing the overall degree of agreement, the McNemar test for paired categorical data (for two categories) or an exact marginal homogeneity test (for 3 ordered categories) will be used to demonstrate the degree of balance in the discordant results. For the fraction of concordant disease status classifications and the overall response rate 95% confidence intervals will be calculated.

For the Re-Treatment arm, statistical analyses will largely mirror that of the initial Treatment arm. The basic statistical analysis plan for the Re-Treatment arm of this proof-of-concept study will describe the subject population by Re-Treatment baseline characteristics: clinical, Quality of Life and pain scores at Re-Treatment baseline. Descriptive summaries will be prepared to show Re-Treatment sample size, mean, standard deviation, 95% confidence intervals (CIs) on the mean, median, minimum, and maximum for continuous variables and counts, percentages, and 95% CIs on the percentage for categorical variables. Time-to-event analyses will be performed with reference to the date of first Re-Treatment on this study. Analyses will focus on response rate and disease stability rate of Re-Treatment and will be largely descriptive for the expected smaller sample size in the Re-Treatment arm within each cohort. Changes in tumor volumes, etc. will be summarized by medians, ranges, and the corresponding 95% CI. This will be done for each cohort separately. For endpoints relating to tumor control and subject well-being, analyses will be done based on the full-analysis, responding, or evaluable data sets, as appropriate. Comparison

against the hypothesized control rate will be done using exact tests for a binomial with the full cohort and then by strata. Based on the safety analysis set, information regarding study treatment administration, drug dosing and course compliance, safety variables, will be described and summarized. Safety will be assessed via tabulations of AEs and SAEs as well as Diary recordings following treatment dose/course of therapy.

12.4 Definitions of Evaluable

12.4.1 Evaluable for Toxicity

12.4.1.1 Any subject who received ≥ one dose of binimetinib and had a ≥ Grade 3 binimetinib-associated toxicity is evaluable for toxicity.

12.4.1.2 In the absence of a ≥ Grade 3 toxicity, any subject who completed one full course of therapy is evaluable for toxicity.

12.4.2 Evaluable for Response – Subjects who have completed at least two courses of therapy and have had their first follow-up MRI evaluation. Subjects who did not respond and are later found to have a target tumor other than a plexiform neurofibroma (e.g. malignant peripheral nerve sheath tumor) are not evaluable for response.

12.4.3 Evaluable for Plasma Cytokines/Growth Factors – Any subject who has at least one sample drawn for plasma cytokines/growth factors prior to starting therapy plus at least one other sample drawn is evaluable for plasma cytokines/growth factors.

13.0 RESPONSE CRITERIA

For initial treatment, response is assessed by the NCI-POB at the time that follow-up 3D-MRI scans are performed (after course 4, 8, 12, and then after completion of every 6 courses thereafter), until disease progression or end of treatment, whichever occurs first. For the purpose of determining the level of response (complete, partial, etc.) measurements from the follow-up scans are compared to the target lesion size in the pretreatment MRI scan using 3D data analysis.

For the Re-Treatment Arm, response is assessed after course 4, 8, 12, and then after completion of every 6 courses thereafter, until disease progression or end of treatment, whichever occurs first. For the purpose of determining the level of response (complete, partial, etc.) measurements from the follow-up scans are compared to the target lesion size in the MRI scan at the time of pre-Re-treatment using 3D data analysis.

Complete Response (CR): A complete resolution of the target plexiform neurofibroma for ≥ 4 weeks

Partial Response (PR): A ≥20% reduction in the volume of the target plexiform neurofibroma lesion for ≥4 weeks.

Stable Disease (SD): A <20% increase, and < 20% decrease in the volume of the target plexiform neurofibroma lesion for ≥4 weeks.

Progressive Disease (PD): A ≥ 20% increase in the volume (by 3D-MRI) of the target plexiform neurofibroma compared to the pretreatment volume.

The appearance of new discrete subcutaneous neurofibromas does not qualify for disease progression.

Worsening of existing symptoms or the appearance of new symptoms that persist for more than 7 days and that are felt to be definitely related to the plexiform neurofibroma should be evaluated by repeating the MRI. Subjects should not be classified as having progressive disease solely on the basis of new or increased symptoms without discussing the case with the protocol Principal Investigator.

Subjects with other evidence of disease progression than outlined above should also be discussed with the Principal Investigator.

14.0 HUMAN SUBJECTS PROTECTION & DATA SAFETY MONITORING PLAN

Rationale for Subject Selection

Neurofibromatosis type 1 is a genetic disorder and the incidence of the disease in the various racial and ethnic groups may vary. This may impact on our ability to recruit sufficient numbers of subjects within each group to this trial. Subject accrual regarding gender, and racial and ethnic groups is described in Section 4.0. None of these groups are excluded from participation in the trial. Females who are pregnant or breast feeding will not be eligible for entry onto the trial because of the potential risks that binimetinib could pose to the fetus or newborn. This trial is designed to determine the activity of binimetinib in children and adults with NF1 and inoperable PN and therefore, subjects <18 years old will be entered onto this research trial. Individuals will be enrolled at one of the sites that participate in the NF consortium as well as PNOC sites. Subjects may also be referred from outside centers but treatment will be directed at an NF or PNOC consortium site.

Participation of Subjects < 18 years of age

The primary endpoint of this trial is to determine whether binimetinib can result in tumor shrinkage in children and adults with PN. Therefore children ≥1 year of age who meet eligibility criteria for this trial will be entered in the study.

Evaluation of benefits and risks/discomforts

The potential benefit from participation in this trial is the stabilization or reduction in the size of the PN, relief of symptoms caused by the PN, and prolongation of life. The primary risk to the subjects from participation in this trial is from binimetinib toxicity (Section 6.1.4). Toxicities from binimetinib are outlined in Section 6.1.4 and are typically reversible when they occur. Subjects enrolled on this trial will be carefully monitored for the development of toxicities, and guidelines for discontinuation of drug and a toxicity stopping rule are in place. Blood samples will be obtained on this trial for optional pharmacodynamics studies. Samples will be identified by a code number that can be traced to the subject only by contacting the trial coordinating center. However, as the blood samples are linked to the subject's name, a small risk persists that unauthorized persons could gain access to information. Some testing may eventually reveal information that could result in discrimination with health or life insurance or employment. We believe that these risks are minimal since it is already known that subjects enrolled on the study have neurofibromatosis. All research results will be kept confidential. Results from the pharmacodynamic studies will be considered preliminary and will require further analysis for verification. Therefore, results will not be communicated with the research subjects. Subjects also have the right to withdraw the blood obtained for research purposes at any time.

Risks to subjects from banking biological specimens include the risks of the blood draws for research purposes. Tumor specimens will be taken only from leftover specimens that were obtained for clinical purposes. The amount of blood to be obtained is minimal (approximately 10 ml) and no more than 5 mL/kg will be taken from any subject in a single day. Risks associated with blood draw are pain, bleeding or bruising at the spot where the needle is inserted, and rarely fainting or infection. Every attempt to draw the research blood samples in conjunction with other clinical blood draws will be made so that the risk of infection is not increased. Blood draws will be performed by trained personnel who are experienced in

working with children and familiar with the challenges of drawing blood from this population. The risk of biological specimen banking is minimized by coding the specimens prior to shipping to the biorepository in order to prevent identification of subjects and protect confidentiality. In addition, the study subject ID number linked to the specimen will be maintained separately at the NF Consortium Operations Center at UAB.

Depending on the age of the subject MRI studies may require sedation or anesthesia, which is associated with additional risks. Consent will be obtained from subjects for sedation or anesthesia for MRI scans as per institutional guidelines.

Risk/benefit analysis

The primary objective of this phase II trial is to define the objective response rate of PN treated with binimetinib, and thus subjects entered on the trial will be treated with therapeutic intent and response to the therapy will be closely monitored. Therefore, this protocol involves greater than minimal risk, but presents the potential for direct benefit to individual subjects. The potential benefit of this treatment with binimetinib is that it may stop or slow down the growth of PN or shrink PN. In addition, binimetinib may lessen the symptoms, such as pain, that are caused by the tumor. The MRI data in this study will be analyzed by a special approach called "volumetric MRI," in addition to being read in a standard manner by a radiologist. The volumetric MRI approach will provide more precise measurement of the size of PN, and therefore will give more complete and objective information on which to base any possible future treatment decisions. Volumetric MRI is currently not available on a routine clinical basis.

Consent and assent process and documentation

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the subject and the subject's parents or guardian if he/she is a child, and a signed informed consent document will be obtained. Consent will be obtained by the site PI or an associate investigator on the trial according to state and institutional guidelines. Age-appropriate assent forms for children have been developed for this trial. This is a multi-institutional trial, and the NF Operations Center will require evidence of local IRB approval and of USAMRMC ORP HRPO approval of the protocol prior to allowing for accrual of subjects at that institution. This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

Research Monitor and Data Safety Monitoring Plan

The Study Chairs and Operations Center clinical research nurse manager will review study progress and safety issues regularly with the NF Operations Center Research Trial Manager. Subject eligibility, deviations and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol. Monthly reports will be generated by the NF Consortium Operations Center to assess completeness of data. There will be monthly phone conferences between the NF Consortium Operations Center and the Protocol Team to address QA issues. A Data Safety Monitoring Board and a Research Monitor for this study will be established for the purpose of ensuring data compliance and regular monitoring.

We will assign a research monitor for this study. He/she will be a qualified physician and is not associated with this particular protocol. He/she will work closely with the Principal Investigator to monitor the participants' treatment while on this study.

The research monitor's duties should be based on specific risks or concerns about the research. The research monitor may perform oversight functions and report their observations and findings to the IRB or a designated official. The research monitor may be identified from within or outside the PI's institution.

Research Monitor functions may include:

- observing recruitment and enrollment procedures and the consent process for individuals, groups or units,
- overseeing study interventions and interactions,
- reviewing monitoring plans and UPIRTSO reports;
- overseeing data matching, data collection, and analysis

There may be more than one research monitor (e.g., if different skills or experiences are necessary). The monitor may be an ombudsman or a member of the data safety monitoring board.
At a minimum, the research monitor:

- may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research;
- shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;
- shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

In addition, on this study, the research monitor is specifically required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator.

15.0 REFERENCES

1. Wu, J., et al., *Plexiform and dermal neurofibromas and pigmentation are caused by Nf1 loss in desert hedgehog-expressing cells.* Cancer Cell, 2008. **13**(2): p. 105-16.
2. Citak, E.C., et al., *Management of plexiform neurofibroma with interferon alpha.* Pediatr Hematol Oncol, 2008. **25**(7): p. 673-8.
3. Kebudi, R., F.B. Cakir, and O. Gorgun, *Interferon-alpha for unresectable progressive and symptomatic plexiform neurofibromas.* J Pediatr Hematol Oncol, 2013. **35**(3): p. e115-7.
4. Jakacki, R.I., et al., *Phase I trial of pegylated interferon-alpha-2b in young patients with plexiform neurofibromas.* Neurology, 2011. **76**(3): p. 265-72.
5. Widemann, B.C., et al., *Phase 1 trial and pharmacokinetic study of the farnesyl transferase inhibitor tipifarnib in children and adolescents with refractory leukemias: a report from the Children's Oncology Group.* Pediatr Blood Cancer, 2011. **56**(2): p. 226-33.
6. Widemann, B.C., et al., *Phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor tipifarnib in children with refractory solid tumors or neurofibromatosis type I and plexiform neurofibromas.* J Clin Oncol, 2006. **24**(3): p. 507-16.
7. Babovic-Vuksanovic, D., et al., *Phase II trial of pirfenidone in adults with neurofibromatosis type 1.* Neurology, 2006. **67**(10): p. 1860-2.
8. Babovic-Vuksanovic, D., et al., *Phase I trial of pirfenidone in children with neurofibromatosis 1 and plexiform neurofibromas.* Pediatr Neurol, 2007. **36**(5): p. 293-300.
9. Kim, A., et al., *Phase I trial and pharmacokinetic study of sorafenib in children with neurofibromatosis type I and plexiform neurofibromas.* Pediatr Blood Cancer, 2013. **60**(3): p. 396-401.
10. Lasater, E.A., et al., *Nf1+/- mice have increased neointima formation via hyperactivation of a Gleevec sensitive molecular pathway.* Hum Mol Genet, 2008. **17**(15): p. 2336-44.
11. McCormick, F., *Ras signaling and NF1.* Curr Opin Genet Dev, 1995. **5**(1): p. 51-5.
12. Sherman, L.S., et al., *Single cell Ras-GTP analysis reveals altered Ras activity in a subpopulation of neurofibroma Schwann cells but not fibroblasts.* J Biol Chem, 2000. **275**(39): p. 30740-5.
13. Downward, J., *Targeting RAS signalling pathways in cancer therapy.* Nat Rev Cancer, 2003. **3**(1): p. 11-22.
14. Weiss, B., G. Bollag, and K. Shannon, *Hyperactive Ras as a therapeutic target in neurofibromatosis type 1.* Am J Med Genet, 1999. **89**(1): p. 14-22.
15. Wu, J., et al., *Preclincial testing of sorafenib and RAD001 in the Nf(flox/flox) ;DhhCre mouse model of plexiform neurofibroma using magnetic resonance imaging.* Pediatr Blood Cancer, 2012. **58**(2): p. 173-80.
16. Kissil, J.L., et al., *What's new in neurofibromatosis? Proceedings from the 2009 NF Conference: new frontiers.* Am J Med Genet A, 2010. **152A**(2): p. 269-83.
17. Yang, F.C., et al., *Nf1-dependent tumors require a microenvironment containing Nf1+/- and c-kit-dependent bone marrow.* Cell, 2008. **135**(3): p. 437-48.

18. Jessen, W.J., et al., *MEK inhibition exhibits efficacy in human and mouse neurofibromatosis tumors.* J Clin Invest, 2013. **123**(1): p. 340-7.
19. Chang, T., et al., *Sustained MEK inhibition abrogates myeloproliferative disease in Nf1 mutant mice.* J Clin Invest, 2013. **123**(1): p. 335-9.
20. Dombi, E., et al., *Activity of Selumetinib in Neurofibromatosis Type 1-Related Plexiform Neurofibromas.* N Engl J Med, 2016. **375**(26): p. 2550-2560.
21. Rosser, T. and R.J. Packer, *Neurofibromas in children with neurofibromatosis 1.* J Child Neurol, 2002. **17**(8): p. 585-91; discussion 602-4, 646-51.
22. Le, L.Q. and L.F. Parada, *Tumor microenvironment and neurofibromatosis type I: connecting the GAPs.* Oncogene, 2007. **26**(32): p. 4609-16.
23. Prada, C.E., et al., *Neurofibroma-associated macrophages play roles in tumor growth and response to pharmacological inhibition.* Acta Neuropathol, 2013. **125**(1): p. 159-68.
24. Wolkenstein, P., et al., *Quality-of-life impairment in neurofibromatosis type 1: a cross-sectional study of 128 cases.* Arch Dermatol, 2001. **137**(11): p. 1421-5.

APPENDIX IA: SCHEDULE OF EVALUATIONS FOR INITIAL TREATMENT

(Please refer to section 9.0 for details)

STUDIES TO BE OBTAINED	Pre-Study[1]	Course 1	Subsequent courses	Completion of Rx
Informed Consent or Assent	X			
Eligibility Checklist	X			
PN Location and Morbidity checklist (see Appendix X)	X			
History	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Physical Exam with vital signs (including T, P, RR, BP)	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Neurological exam	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Performance Status (see Appendix II)	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Ophthalmological toxicity exam [3]	X		Post course 4, 8, 12, 18, 24 †	X
Binimetinib Diary Review		End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
CBC, differential, platelets	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Electrolytes and blood chemistries including Na, K, Cl, bicarb, BUN, Cr, glucose, Ca, phosphorous, albumin, total protein, alk phos, SGOT, SGPT, bilirubin, CPK	X	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Urine/Serum Pregnancy[4]	X (within 7 days prior)	End†	Post course 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
ECHO (or MUGA) and ECG	X (within 4 weeks prior)		Post course 4, 8, 12, 18, 24 †	X
MRI including volumetric analysis	X (within 4 weeks prior)		Post course 4, 8, 12, 18, 24 †	X[5]
Functional Evaluation (see Appendix XII-XV)	X (within 4 weeks prior)		Post course 4, 8, 12, 18, 24 †	X
Photography of Visible PN (see Appendix XVI)	X (within 4 weeks prior)		Post course 4, 8, 12, 18, 24 †	X
PN Clinician Morbidity Assessment (see Appendix XI)	X		Post course 4, 8, 12, 18, 24 †	X
PN Symptom checklist (see Appendix IX)	X		Post course 4, 8, 12, 18, 24 †	X
Health-related quality of life and pain assessments	X		Post course 4, 8, 12, 18, 24 †	X
Cytokine studies (see Appendix VII)	X	end	Post course 4	
Blood and Tissue collection[6] (see Appendix XXI)	X		Post course 4 and 12	X

† See section 9 for definitions of end of course.

1. All studies to determine eligibility must be performed within 2 weeks prior to enrollment unless otherwise indicated below (See section 9.0). Consent must be obtained prior to enrollment but does not expire at 2 weeks.

2. Height only required at baseline

3. Ophthalmology toxicity evaluation: Eye exam with emphasis on corneal, lens and retinal changes. In subjects with orbital plexiform neurofibromas the intraocular pressure should be measured at baseline and subsequently if feasible.

4. For females of childbearing age

5. Subjects who had a response (≥20% target lesion shrinkage by 12 courses) must have an MRI performed at 4 months and 12 months after stopping drug if they are still on study. Of note these MRIs can be done at a local institution but needs to be submitted by the enrolling site,

6. Blood samples should be drawn at the indicated time points as well as around the time of tumor biopsy if tumor tissue is being submitted (if the patient undergoes a clinically indicated tissue collection procedure). The following will be collected at each time point: Streck tube (for plasma): ~ 2 mL; EDTA tube (for DNA): ~ 5 mL; PAXGene tube (for RNA) ~ 2.5 mL.

APPENDIX IB: SCHEDULE OF EVALUATIONS FOR RE-TREATMENT

(Please refer to section 9.0 for details)

STUDIES TO BE OBTAINED	Pre-Study[1]	Course 1	Subsequent courses[5]	Completion of Rx
Informed Consent or Assent	X			
Eligibility Checklist	X			
PN Location and Morbidity checklist (see Appendix X)	X			
Phone Call (to assess drug compliance and toxicity)		End†		
History	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Physical Exam with height, weight, and vital signs (including T, P, RR, BP)[2]	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Neurological exam	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Performance Status (see Appendix II)	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Ophthalmological toxicity exam[3]	X (within 4 weeks prior)		Post course 6, 12, 18, 24 †	X
Binimetinib Diary Review			Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
CBC, differential, platelets	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Electrolytes and blood chemistries including Na, K, Cl, bicarb, BUN, Cr, glucose, Ca, phosphorous, albumin, total protein, alk phos, SGOT, SGPT, bilirubin, CPK	X		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
Urine/Serum Pregnancy[4]	X (within 7 days prior)		Post course 2, 4, 6, 8, 10, 12, 15, 18, 21, and 24 †	X
ECHO (or MUGA) and ECG	X (within 4 weeks prior)		Post course 6, 12, 18, 24 †	X
MRI including volumetric analysis	X (within 4 weeks prior)		Post course 4, 8, 12, 18, 24 †	X
PN Clinician Morbidity Assessment (see Appendix XI)	X		Post course 4, 8, 12, 18, 24 †	X
PN Symptom checklist (see Appendix IX)	X		Post course 4, 8, 12, 18, 24 †	X
Health-related quality of life and pain assessments	X		Post course 4, 8, 12, 18, 24 †	X

† See section 9 for definitions of end of course.

1. All studies to determine eligibility must be performed within 2 weeks prior to enrollment except for MRI, ophthalmology, Echo, and EKG (which is required within 4 weeks prior to "enrollment" on the Re-Treatment Arm), unless otherwise indicated. Consent must be obtained prior to enrollment but does not expire at 2 weeks. Re-Treatment eligibility studies may be counted as the Re-Treatment baseline studies as long as there has been no concerning change in clinical status prior to starting study drug, except for pregnancy testing in females of childbearing age which must be negative within 7 days prior to re-starting binimetinib.
2. Height required only at baseline
3. Ophthalmology toxicity evaluation: Eye exam with emphasis on corneal, lens and retinal changes. In subjects with orbital plexiform neurofibromas the intraocular pressure should be measured at baseline and subsequently if feasible.
4. For females of childbearing age
5. For participants staying on study while arrangements are being made to transition the participant to a MEK inhibitor off study (see section 6.2), evaluations should occur as per protocol section 9.

APPENDIX II: PERFORMANCE STATUS SCALES/SCORES

Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	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.
50	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.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX III: NIH CONSENSUS CLINICAL DEFINTION OF NEUROFIBROMATOSIS

- Six or more café-au-lait spots (≥0.5 cm in prepubertal subjects or ≥1.5 cm in postpubertal subjects)
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axilla or groin
- Optic glioma
- Two or more Lisch nodules
- A distinctive bony lesion (dysplasia of the sphenoid bone or dysplasia or thinning of long bone cortex)
- A first-degree relative with NF1

APPENDIX IV: PROTOCOL FOR REQUIRED PRE-STUDY AND ON-STUDY MRI STUDIES

Prior to starting treatment, a measurable target plexiform neurofibroma must be identified. In addition, up to two non-target plexiform neurofibromas may be identified as well.

The goal will therefore be to use 3-D MRI only to follow the target and non-target plexiform neurofibroma(s) (a maximum of three lesions), which will be defined as index lesion(s).

Pre-study imaging evaluation:

1. Identify and select the index plexiform neurofibroma(s) (a maximum of three lesions) for 3-D MRI evaluation based on prior imaging studies. Should there be more than 3 progressing plexiform neurofibromas; the three most clinically relevant plexiform neurofibromas should be followed by 3-D MRI analysis.
2. Perform 3-D MRI sequences on the selected index lesions as outlined in the MRI acquisition protocols below.

On study imaging evaluation:

Unless clinically indicated otherwise obtain MRI of the index lesion(s) (target and non-target) only as outlined in the MRI acquisition protocol below after course 4, 8, 12, 18, and 24 while on study.

MRI protocols:

Images intended for volumetric analysis need to be performed without gaps between slices. Every attempt should be made to image the entire PN. The target tumor should be positioned close to the center of the imaging field and the outer edge of the tumor should be within the field of view. Peripheral nerve sheath tumors can be well visualized without the use of contrast agents on short TI inversion recovery (STIR) sequences because they have high signal intensity relative to normal tissues. The imaging protocol in the table below should be used for the imaging of the target and non-target PN.

If necessary, participating institutions may modify the MRI sequences to optimize differentiation of tumor and surrounding tissue. Modifications should be noted, such that the same imaging protocol, and, if possible, the same MRI scanner, can be used for all subsequent MRI studies.

All MRI studies requested per protocol will be submitted to the NCI POB within 1 week of acquisition for volume analysis.

Volumetric sequence for PN**			
Axial STIR	**Recommended Range**	**Head-Neck or Small[##] Trunk-Extremity PN**	**Large[##] Trunk-Extremity PN**
Echo Train Length	5 - 15	7	15
TR	3000 - 6000	6000	4000

TE	30 - 50	34	30
TI	150-180	150	150
Slice Thickness	3 - 10 mm	3 mm	10 mm
Skip	0	0	0
Matrix	256x256 - 512x512	256x256	320 x256 - 512x512
FOV	18 - 50 cm	22 cm	45 cm
Phase FOV	0.8	0.8	0.8
NEX	2 - 4	3	2
Frequency Direction	A→P	A→P	A→P

**Spinal/paraspinal PN should be imaged with the appropriate protocol based on tumor size. Tumors that include the neck and trunk, for which the majority of the tumor is in the trunk, should be imaged with the Large Trunk-Extremity PN protocol.
##Tumors < 5 cm in longest diameter would be considered small. Tumors > 10 cm longest diameter would be considered large. Tumors between 5-10 cm in longest diameter should be imaged with the appropriate protocol based on best FOV coverage.
Please contact Dr. Dombi if there are questions regarding the appropriate coverage or imaging protocol.

Data Analysis:

- All MRI data will be analyzed at the Pediatric Oncology Branch of the NCI. The MRI data from each scan will be processed to assess the volume of the index plexiform neurofibroma. The tumor will be traced on subsequent contiguous MR slices, the numbers summed and then multiplied by the slice thickness to obtain a numerical volume measurement. The tumor will be identified by high signal on the STIR images not corresponding to known normal anatomic structures and corresponding with the course of known nerves. Each subject's volumetric measurement obtained from the initial MRI will serve as the baseline against which to assess incremental changes in volume that occur during the subsequent intervals. Volumetric measurements and 1-D, and 2-D data analysis will be done by 3 physicians trained in 1-D, 2-D, and 3-D MRI data analysis at the Pediatric Oncology Branch, NCI at an Image Review Workstation using MEDx software (Sensor Systems Inc.). Volumetric measurements will be used to determine disease progression as outlined in Section 13. Drs. Dombi and Widemann will inform Participating Investigators, the Principal Investigator, and the NF Consortium Operations Center about the results of the MRI study by written report.

Image and Data Acquisition:

- In order to perform quantitative analysis, the Pediatric Oncology Branch must receive the imaging data from the investigator sites. All MRI studies requested per protocol will be submitted to the NCI POB within 1 week of acquisition for volume analysis. The Pediatric Oncology Branch will check all materials received for completeness and will notify the site if data, images, or information are missing

or incomplete. Dr. Dombi will confirm for each patient in screening that the MRI imaging is adequate and that the target PN is measurable and amenable to volumetric MRI analysis.

- Address for shipment of imaging studies:
  Dr. Eva Dombi, MD
  NCI, POB
  10 Center Drive, Building 10, room 1-5750
  Bethesda, MD 20892-1101
  Phone 301-451-7023,
  e-mail: dombie@mail.nih.gov
  fax: 301-402-1734

APPENDIX IVa: MRI ELIGIBILITY FORM

This form must accompany all MRI studies sent to the NCI POB for review of target plexiform neurofibroma eligibility (i.e. whether it is measurable and amenable to volumetric analysis). Please mail the MRI on a CD along with this form to:

Eva Dombi, MD
NCI, POB
10 Center Drive, Building 10 CRC, Room 1-5750
Bethesda, MD 20892
Phone: 301-451-7023
E-mail: dombie@mail.nih.gov

PID#1:_____ Date of MRI: _____

Location of target plexiform: _____

From:
Name of Investigator: _____

Study Center: _____

Phone #: _____

Email Address: _____

_____
From Eva Dombi, MD:

The target PN is measurable and amenable to volumetric analysis:

Yes:_____ No: _____

Comments: _____

_____

_____

APPENDIX V: SUBJECT DIARY ON BINIMETINIB PROTOCOL

* For patients taking binimetinib in tablet form *

Patient Name: _____ **Drug:**

Start Date : ____/____/ ____ **End Date : ____/ ____/ ____** **Dose: mg**

Instructions:

1) *Record the date and time (circle AM or PM) for each daily dose and number of tablets of binimetinib taken*
2) *Binimetinib should be taken at approximately the same time each day, with or without food*
3) *If you miss a dose (e.g., due to vomiting), do not take another dose. Resume dosing with the next scheduled dose.*
4) *Please enter the information for this diary as it occurs daily.*

	Date	Dose taken	# of tablets taken	Dose taken	# of tablets taken	Comments
Day 1		: AM		: PM		
Day 2		: AM		: PM		
Day 3		: AM		: PM		
Day 4		: AM		: PM		
Day 5		: AM		: PM		
Day 6		: AM		: PM		
Day 7		: AM		: PM		
Day 8		: AM		: PM		
Day 9		: AM		: PM		
Day 10		: AM		: PM		
Day 11		: AM		: PM		
Day 12		: AM		: PM		
Day 13		: AM		: PM		
Day 14		: AM		: PM		
Day 15		: AM		: PM		
Day 16		: AM		: PM		

Day 17		: AM		: PM		
Day 18		: AM		: PM		
Day 19		: AM		: PM		
Day 20		: AM		: PM		
Day 21		: AM		: PM		
Day 22		: AM		: PM		
Day 23		: AM		: PM		
Day 24		: AM		: PM		
Day 25		: AM		: PM		
Day 26		: AM		: PM		
Day 27		: AM		: PM		
Day 28		: AM		: PM		

* For patients taking liquid suspension of binimetinib *

Patient Name: _____ **Drug:**

Start Date : ____/____/ ____ **End Date : ____/ ____/ ____** **Dose: mg**

Instructions:

5) *Record the date and time (circle AM or PM) for each daily dose and number of mLs of binimetinib taken*
6) *Binimetinib should be taken at approximately the same time each day, with or without food*
7) *If you miss a dose (e.g., due to vomiting), do not take another dose. Resume dosing with the next scheduled dose.*
8) *Please enter the information for this diary as it occurs daily.*

Date		Dose taken	# of mLs	Dose taken	# of mLs	Comments
Day 1		: AM		: PM		
Day 2		: AM		: PM		
Day 3		: AM		: PM		
Day 4		: AM		: PM		
Day 5		: AM		: PM		
Day 6		: AM		: PM		
Day 7		: AM		: PM		
Day 8		: AM		: PM		
Day 9		: AM		: PM		
Day 10		: AM		: PM		
Day 11		: AM		: PM		
Day 12		: AM		: PM		
Day 13		: AM		: PM		
Day 14		: AM		: PM		
Day 15		: AM		: PM		
Day 16		: AM		: PM		
Day 17		: AM		: PM		

Day 18		: AM		: PM		
Day 19		: AM		: PM		
Day 20		: AM		: PM		
Day 21		: AM		: PM		
Day 22		: AM		: PM		
Day 23		: AM		: PM		
Day 24		: AM		: PM		
Day 25		: AM		: PM		
Day 26		: AM		: PM		
Day 27		: AM		: PM		
Day 28		: AM		: PM		

APPENDIX VI: INFORMATION ON POSSIBLE DRUG INTERACTIONS

List of inhibitors and inducers of UGT1A1 to be used with caution

Inhibitors of UGT1A1	Inducers of UGT1A1
Atazanavir, erlotinib, flunitrazepam, gemfibrozil, indinavir, ketoconazole, nilotinib, pazopanib, propofol, regorafenib, sorafenib	Carbamazepine, nicotine, rifampicin, testosterone propionate

Table VI - 1 List of drugs with a conditional risk of Torsades de Pointes

Generic Name	Brand Name	Class/Clinical Use
Amisulpride	Solian® and others	Antipsychotic, atypical / schizophrenia
Amitriptyline	Elavil®	Tricyclic Antidepressant / depression
Ciprofloxacin	Cipro®	Antibiotic / bacterial infection
Clomipramine	Anafranil®	Tricyclic Antidepressant / depression
Desipramine	Pertofrane®	Tricyclic Antidepressant / depression
Diphenhydramine	Benadryl®, Nytol®	Antihistamine / Allergic rhinitis, insomnia
Doxepin	Sinequan®	Tricyclic Antidepressant / depression
Fluconazole	Diflucan®	Anti-fungal / fungal infection
Fluoxetine	Sarafem®, Prozac®	Anti-depressant / depression
Galantamine	Reminyl®	Cholinesterase inhibitor / Dementia, Alzheimer's
Imipramine	Norfranil®	Tricyclic Antidepressant / depression
Itraconazole	Sporanox®	Anti-fungal / fungal infection
Ketoconazole	Nizoral®	Anti-fungal / fungal infection
Nortriptyline	Pamelor®	Tricyclic Antidepressant / depression
Paroxetine	Paxil®	Anti-depressant / depression
Protriptyline	Vivactil®	Tricyclic Antidepressant / depression
Ritonavir	Norvir®	Protease inhibitor / HIV
Sertraline	Zoloft®	Anti-depressant / depression
Solifenacin	VESIcare®	muscarinic receptor anatagonist / treatment of overactive bladder
Trazodone	Desyrel®	Anti-depressant / Depression, insomnia
Trimethoprim-Sulfa	Septra® or Bactrim®	Antibiotic / bacterial infection
Trimipramine	Surmontil®	Tricyclic Antidepressant / depression
Substantial evidence supports the conclusion that these drugs prolong the QT interval and have a risk of TdP but only under certain known conditions (e.g. excessive dose, drug interaction, etc.).		

Table VI - 2 List of drugs with a possible risk of Torsades de Pointes

Generic Name	Brand Name	Class/Clinical Use
Alfuzosin	Uroxatral®	Alpha1-blocker / Benign prostatic hyperplasia
Amantadine	Symmetrel®	Dopaminergic/Anti-viral / Anti-infective/ Parkinson's Disease
Artenimol+piperaquine	Eurartesim®	Anti-malarial / malaria infection
Atazanavir	Reyataz®	Protease inhibitor / HIV
Chloral hydrate	Noctec®	Sedative / sedation/ insomnia
Clozapine	Clozaril®	Anti-psychotic / schizophrenia
Dolasetron	Anzemet®	Anti-nausea / nausea, vomiting
Dronedarone	Multaq®	Anti-arrhythmic / Atrial Fibrillation

Generic Name	Brand Name	Class/Clinical Use
Eribulin	Halaven®	Anti-cancer / metastatic breast neoplasias
Escitalopram	Cipralex®, Lexapro®	Anti-depressant / Major depression/ Anxiety disorders
Famotidine	Pepcid®	H2-receptor antagonist / Peptic ulcer/ GERD
Felbamate	Felbatrol®	Anti-convulsant / seizure
Fingolimod	Gilenya®	Immunosuppressant / Multiple Sclerosis
Foscarnet	Foscavir®	Anti-viral / HIV infection
Fosphenytoin	Cerebyx®	Anti-convulsant / seizure
Gatifloxacin	Tequin®	Antibiotic / bacterial infection
Gemifloxacin	Factive®	Antibiotic / bacterial infection
Granisetron	Kytril®	Anti-nausea / nausea and vomiting
Iloperidone	Fanapt®	Antipsychotic, atypical / Schizophrenia
Indapamide	Lozol®	Diuretic / stimulate urine & salt loss
Isradipine	Dynacirc®	Anti-hypertensive / high blood pressure
Lapatinib	Tykerb®, Tyverb®	Anti-cancer / breast cancer, metastatic
Levofloxacin	Levaquin®	Antibiotic / bacterial infection
Lithium	Lithobid®, Eskalith®	Anti-mania / bipolar disorder
Moexipril/HCTZ	Uniretic®	Anti-hypertensive / high blood pressure
Nicardipine	Cardene®	Anti-hypertensive / high blood pressure
Nilotinib	Tasigna®	Anti-cancer / Leukemia
Octreotide	Sandostatin®	Endocrine / acromegaly, carcinoid diarrhea
Ofloxacin	Floxin®	Antibiotic / bacterial infection
Ondansetron	Zofran®	Anti-emetic / nausea and vomiting
Oxytocin	Pitocin®	Oxytocic / Labor stimulation
Paliperidone	Invega®	Antipsychotic, atypical / Schizophrenia
Perflutren lipid microspheres	Definity®	Imaging contrast agent / Echocardiography
Quetiapine	Seroquel®	Anti-psychotic / schizophrenia
Ranolazine	Ranexa®	Anti-anginal / chronic angina
Risperidone	Risperdal®	Anti-psychotic / schizophrenia
Roxithromycin*	Rulide®	Antibiotic / bacterial infection
Sertindole	Serdolect®, Serlect®	Antipsychotic, atypical / Anxiety, Schizophrenia
Sunitinib	Sutent®	Anti-cancer / RCC, GIST
Tacrolimus	Prograf®	Immunosuppressant / Immune suppression
Tamoxifen	Nolvadex®	Anti-cancer / breast cancer
Telithromycin	Ketek®	Antibiotic / bacterial infection
Tizanidine	Zanaflex®	Muscle relaxant / spasticity
Vardenafil	Levitra®	phosphodiesterase inhibitor / vasodilator
Venlafaxine	Effexor®	Anti-depressant / depression
Voriconazole	VFend®	Anti-fungal / anti-fungal
Ziprasidone	Geodon®	Anti-psychotic / schizophrenia
Substantial evidence supports the conclusion that these drugs cause QT prolongation but there is insufficient evidence that they, when used as directed in labeling, have a risk of causing TdP.		

Table VI - 3 List of drugs with a known risk of Torsades de Pointes

Generic Name	Brand Name	Class/Clinical Use
Amiodarone	Cordarone®, Pacerone®	Anti-arrhythmic / abnormal heart rhythm
Arsenic trioxide	Trisenox®	Anti-cancer / Leukemia
Astemizole	Hismanal®	Antihistamine / Allergic rhinitis
Azithromycin	Zithromax®	Antibiotic / bacterial infection
Bepridil	Vascor®	Anti-anginal / heart pain
Chloroquine	Aralen®	Anti-malarial / malaria infection
Chlorpromazine	Thorazine®	Anti-psychotic/ Anti-emetic / schizophrenia/ nausea
Cisapride	Propulsid®	GI stimulant / heartburn
Citalopram	Celexa®	Anti-depressant / depression
Clarithromycin	Biaxin®	Antibiotic / bacterial infection
Disopyramide	Norpace®	Anti-arrhythmic / abnormal heart rhythm
Dofetilide	Tikosyn®	Anti-arrhythmic / abnormal heart rhythm
Domperidone	Motilium®	Anti-nausea / nausea
Droperidol	Inapsine®	Sedative; Anti-nausea / anesthesia adjunct, nausea
Erythromycin	E.E.S. ®, Erythrocin®	Antibiotic;GI stimulant / bacterial infection; increase GI motility
Flecainide	Tambocor®	Anti-arrhythmic / abnormal heart rhythm
Halofantrine	Halfan®	Anti-malarial / malaria infection
Haloperidol	Haldol®	Anti-psychotic / schizophrenia, agitation

APPENDIX VII: PHARMACODYNAMIC STUDIES

Plasma Cytokines and Growth Factors
CYTOKINE STUDIES Worksheet
_____ BSA (m^2) Binimetinib dose (mg) ___, if given

Plasma Cytokines and Growth Factors Procedure:
10 ml blood will be drawn into EDTA tubes before treatment initiation, end of course 1 and end of course 4. The sample will be collected at least 30 min but no more than 2 hours after taking the morning dose. To summarize:

- **Pre-study measurement needed**
- **Post Course 1: collection 30 minutes to 2 hours after taking the morning dose**
- **Post Course 4: collection 30 minutes to 2 hours after taking the morning dose**

• After collection, samples should be gently inverted 15 times to completely mix whole blood and anticoagulant
• Blood samples should then be immediately placed into an ice bath so that they are kept at 2-8° Celsius
• Whole blood samples should be centrifuged at 4 °Celsius for 10 minutes at 1,700 x g to separate the plasma from the red blood cells
• Transfer plasma layer using a clean disposable pipette into the appropriately labeled 3.6mL cryovial
• Immediately store plasma sample in a -70 °Celsius freezer until shipment. Once frozen, samples should not be allowed to thaw, including during shipment.
• **Time from blood collection to freezing of plasma should not exceed 30 minutes**

Samples will be shipped on dry ice **Monday through Thursday.**
Samples will be shipped to Cincinnati Children's Hospital Medical Center. Samples should be shipped with express next day priority delivery. Samples will be shipped to the following address:

Cincinnati Children's Hospital Medical Center
S Dock, Ratner Laboratory S7.348
240 Albert Sabin Way
Cincinnati, OH 45229
c/o Nancy Ratner S7.250 Phone: 513-636-3502
Email: Lindsey.Aschbacher-Smith@cchmc.org
Other email contacts in the lab: Nancy.Ratner@cchmc.org; Jianqiang.Wu@cchmc.org;

Please include this completed worksheet with the sample shipment:

Subject ID	
Course	
Course day	
Date of sample	
Date **and** time of last binimetinib dose:	

APPENDIX VIII: CONSORTIUM QOL AND PAIN MEASURES

PEDIATRIC PRO MODULE

(Ages 2 – 17 years)*

I. QOL and Pain Measures for Pediatric Patients

In pediatric participants with plexiform neurofibromas in all locations, this study will assess general health-related qualify of life (QOL) and pain (pain intensity and pain interference) using patient-reported outcomes (PROs) as described below.
*IMPORTANT ADMINISTRATION GUIDELINES: If a participant is 17 years at study entry they should be administered the PROs in the Pediatric Module, continuing on the same pediatric measures even if they turn 18 years old. However, if they are 18 years at study entry, they should be administered the PROs in the Adult Module.

Description of QOL and Pain Measures

General Health-Related Quality of Life
Pediatric Quality of Life Inventory (PedsQL; Varni, 2001): The PedsQL 4.0 Generic Core Scales (Acute Form assessing the past 7 days) are multidimensional child *self-report* and *parent proxy report* scales to assess health-related quality of life (QOL) in children, adolescents, and young adults ages 2 – 25 years. It is a brief standardized pediatric QOL scale with good reliability and validity, which includes both generic and disease specific modules. It consists of a 23-item core measure of global QOL that has four subscales: physical functioning, emotional functioning, social functioning, and school functioning. The domain of physical functioning will be used as a specific secondary outcome measure for this study, especially the parent proxy for younger children as it assesses children under 6 years of age.
There are different forms for parents of patient's ages 2 – 17 years (toddler: 2 – 4; young child: 5 – 7; child: 8 – 12; adolescent: 13 – 17) and parallel self-report forms for patient's ages 5 – 25 years (young child: 5 – 7; child: 8 – 12; adolescent: 13 – 17; young adult: 18 – 25). It takes approximately 5 - 10 minutes to complete. For this study, children from 8 to 17 years of age will complete *self-report* measures of the PedsQL, and parents or legal guardians of children from 2 to 17 years of age will complete the *parent proxy report* measures of the PedsQL.

Pain Intensity
The Numerical Rating Scale-11 (NRS-11) is a *self-report* segmented 11-point numeric scale that assesses pain severity (Hawker et al., 2011). It consists of a horizontal line with 0 representing "no pain" at the right end of the line and 10 representing "worst pain you can imagine" at the left end. Patients are asked to circle the one number from 0 to 10 that best describes their worst pain over the past week for the following: 1) most important tumor pain chosen by the participant, 2) target tumor pain and 2) other pain. It takes less than 5 minute to complete. The NRS-11 is recommended as a core outcome *self-report* measure of pain intensity for clinical trials (Dworkin et al., 2005) in children with NF1 (Wolters, et al., 2013). Children, ages 8 to 17 years, will complete self-report measures of the NRS-11 for this study.

Pain Interference
The Pain Interference Index (PII) is a 6-item measure that assesses the degree to which pain has interfered with daily activities in the past week. This measure was developed in Sweden and validated in Swedish with a group of children and adolescents with longstanding idiopathic pain (Wicksell, Melin, Lekander, & Olsson, 2009). The author (R. Wicksell) translated the measure into English, and members of the Neurobehavioral Group (S. Peron and P. Wolters) worked with the Swedish group to modify the wording

to make it more readable and to shorten the time frame to one week, and we adapted it to create a parallel parent version. Dr. Wicksell and the Swedish research group are supportive of our efforts to validate the self-report and parent versions of the PII in NF1. Results of our validation studies in children with NF1 and their parents indicate that the internal consistency and construct validity of the PII are good. These data support the use of the PII to assess pain interference in youth with NF1 and PNs (Martin et al., submitted). For this study, children from 8 to 17 years of age will complete *self-report* PII, and parents or the legal guardian of children from 5 to 17 years of age will complete the *parent proxy report* PII.

Global Impression of Change

The patient Global Impression of Change (GIC) Scale evaluates the clinical significance of changes in pain intensity. We will administer an adapted version of the *self-report* GIC Scale at the **follow-up** PRO evaluations only (it should not be given pre-study because it assesses change in pain from baseline). A parallel *parent proxy report* form also was developed. On the adapted GIC, patients (and their parents separately) will give their overall impression of change in level of the child's 1) tumor pain, 2) other pain, and 3) other morbidities from before initiation of the MEK inhibitor to the current evaluation point for the first year on study. This global measure of change will be administered to children ≥8 years and for parents of children ≥ 5 years of age at each PRO assessment through the first year (prior to course 13). The GIC Scale has been used in several research studies on chronic pain in adults and more recently children (Arnold et al., 2011; Mohammad et al., 2014). It has been recommended by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) for clinical trials involving chronic pain (Dworkin et al., 2005; McGrath et al., 2008). The relationship between the numerical scale (NRS-11) and the qualitative scale (GIC) will be determined by breaking the participants into groups based on their qualitative response and determining the mean point difference associated with each qualitative label. This information will help evaluate the effects of the study drug on pain as well as provide data on clinically meaningful changes in pain.

Pediatric Background Information

The Pediatric QOL Background Form should be completed by the parents of all patients ages from 2-17 years **at each evaluation** to document information about demographics, use of pain medication, and NF1 disease severity. These data will be used to better interpret the PRO data.

Administration Schedule of the PRO Measures

• Baseline and follow-up PRO assessments: Child participants and their parents will complete the PRO measures at baseline (prior to starting treatment) and then again after course 4 ±2 weeks, 8 ±2 weeks, 12 ±2 weeks, 18 ±2 weeks, and 24 ±2 weeks. In addition, they should be administered at the end of treatment ±2 weeks if this is not encompassed in the times above.

Specific Administration Instructions

• The instructions for each PRO form should be reviewed with the parent and child prior to having them complete it. For children of all ages completing the self-report forms, it is important to have an investigator in the room while the child completes them to review the instructions and answer any questions. Younger children may need help understanding the directions or reading the items.

• Items on the NRS-11 pain intensity scale asking the child to rate their most important tumor pain that they pick and their target tumor pain selected by the medical team. The SAME tumor

pains rated at the baseline evaluation needs to be rated for each subsequent evaluation so the investigator administering the PRO forms needs to write in the tumor pains rated at the baseline evaluation each time.

• Prior to seeing the patients for the **baseline** evaluation, find out the area of the body where the target tumor is located (from the designated investigator at your site) so that you can tell patients this location and write it in the blank space for question #2 on the NRS-11.

• When administering these questionnaires, first review the instructions on each form with the parents and child participants. Mention that you can read or explain any of the items if they need any help reading or if they have any questions.

• For participants who may have difficulty reading, it is permissible to read the items to them, or explain any items they have difficulty understanding, but the patients should answer the items themselves. One way to do this is to give the patient their own form on which to mark his/her answers while the staff person reads the items from another copy of the questionnaire.

• Immediately after the participants are finished filling out the questionnaires and before they leave the clinic, please **check each form** to make sure that the items are completed correctly (e.g., only one response circled) and that all the applicable items are answered.

--If more than one answer is circled for an item, please have the individual choose one response.

--If they forgot to answer an item, ask them to please try to answer it.

• Prior to seeing patients for all of the **follow-up** evaluations, refer back to the completed baseline NRS-11 pain intensity questionnaire to identify the location of the tumor that the participant previously specified as their most important tumor and then write this information on the blank line in question #1 on the current form. Also, write in the target tumor rated at baseline for item #2. Specifying these locations will help ensure that the patients rate the severity of pain for the same tumor across all evaluations.

Self-report

• Children, 8 to 17 years of age, will complete the self-report measures of the PedsQL, NRS-11, and PII at baseline and all follow-up evaluations. The GIC scale will be done only at the follow-up evaluations after the other measures have been completed.

Parent Report

• Parents (or the legal guardian) of children, 2 to 17 years of age, will complete the parent proxy measures of the PedsQL and a background form.

• Parents (or the legal guardian) of children, 5 to 17 years of age, also will complete the parent proxy measures of the PII. At the follow-up evaluations only, they will be administered the GIC scale, which is to be completed after the other measures have been done.

Table 1. PROs administered to <u>all</u> pediatric patients (ages 2 - <18 years)* by age and respondent

<u>Patient</u> Questionnaire	**Age of Patient (years)***			
	2 - 7		8 - 17	
Pain Intensity				
Patient *self-report:* Numeric Rating Scale-11			√	
Pain Interference				
Patient *self-report:* Pain Interference Index			√	
General QOL				
Patient *self-report:* PedsQL Generic form			√ 8 - 12 (Child)	√ 13 - <18 (Teen)
Global Impression of Change				
Patient *self-report:* Global Impression of Change Scale			√ (follow-up evaluations only)	
<u>Parent</u> Questionnaires	**Age of Patient (years)**			
	2 - 7		8 - 17	
Background Information				
Parent *report:* Pediatric Background Form	√		√	
Pain Interference				
Parent *report:* Pain Interference Index		√ (5 - 7)	√	
General QOL				
Parent *report:* PedsQL Generic form	√ 2 - 4 (Toddler)	√ 5 - 7 (Young Child)	√ 8 - 12 (Child)	√ 13 - 17 (Teen)
Global Impression of Change				
Parent *report*: Global Impression of Change Scale		√ (5 - 7; follow-ups evals only)	√ (follow-up evaluations only)	

√ Administer items with a check mark under the correct age range of the patient

* IMPORTANT ADMINISTRATION GUIDELINES: If a participant is 17 years at study entry they should be administered the PROs in the Pediatric Module, continuing on the pediatric measures even if they turn 18 years old. However, if they are 18 years at study entry, they should be administered the PROs in the Adult Module.

ADULT PRO MODULE

(Ages ≥18 years)*

II. QOL and Pain Measures for Adult Patients

In adult participants with plexiform neurofibromas in all locations, this study will assess general health-related qualify of life (QOL) and pain (pain intensity and pain interference) using patient-reported outcomes (PROs) in the Adult Module as described below.

*IMPORTANT ADMINISTRATION GUIDELINES: If a participant is 18 years or older at study entry, they should be administered the PROs in the Adult Module. However, if a participant is 17 years or younger at study entry, they should be administered the PROs in the Pediatric Module, continuing on the same pediatric measures even if they turn 18 years old.

NF1 Disease Specific QOL Measure

The Adult PedsQL™ NF1 Module is a 74-item self-report instrument to assess NF1 disease-specific quality of life (QOL) in adults, ages 18 years and older. It is comprised of 16 scales: 1) Physical functioning (8 items), 2) Emotional functioning (5 items), 3) Social functioning (3 items), 4) Cognitive functioning (5 items), 5) Communication (3 items), 6) Worry (7 items), 7) Perceived physical appearance (3 items), 8) Pain and Hurt (3 items), 9) Paresthesias (2 items), 10) Skin irritation (5 items), 11) Sensation (4 items), 12) Movement and Balance (4 items), 13) Fatigue (3 items), 14) Daily activities (12 items), 15) Treatment anxiety (4 items) and 16) Sexual functioning (3 items) (Nutakki et al., submitted; N. Swigonski, personal communication, September 26, 2012).

The PedsQL™ NF1 Module format, instructions, and Likert response scale are similar to the PedsQL™ 4.0 Generic Core Scales and other PedsQL™ Disease-Specific Modules. The instructions ask how much of a problem each item has been during the past one month. A 5-point response scale is used for all items in the three versions of the instrument (0= never a problem, 1= almost never a problem, 2= sometimes a problem, 3= often a problem, 4= almost always a problem). Items are reverse scored and linearly transformed to a scale of 0-100 similar to PedsQL™ 4.0 Generic Core Scales (0= 100, 1= 75, 2= 50, 3=25, 4=0). Higher scores indicate better HRQOL and fewer symptoms or problems. The total scale score is computed as the sum of all items on the PedsQL™ NF1 Module divided by the number of items answered (this accounts for missing data). Subscale scores are computed as the sum of the items divided by the number of items that were answered in that scale. If more than 50% of the items in the scale are missing, the scale score is not computed.

Feasibility, measured by the percentage of missing responses, was 4.8 % for all scales on the adult version of the PedsQL™ NF1 Module. Internal consistency reliability for the Total Scale score (alpha =0.97) and Scale reliabilities ranging from 0.72 to 0.96 were acceptable for group comparisons. The PedsQL™ NF1 module distinguished between NF1 adults with excellent to very good, good, and fair to poor health status. Thus, preliminary data demonstrates the initial feasibility, reliability and validity of the PedsQL™ NF1 module in adult patients (Nutakki et al.,

personal communication). The preliminary data in a small sample of adolescents also looks promising with good feasibility (percentage of missing responses was 8.9%) and a total scale reliability of 0.96 (N. Swigonski, personal communication, October 10, 2012).

The PlexiQoL was developed as an outcome measure specific to adults with NF1 associated plexiform neurofibromas. Content for the measure was developed directly from qualitative interviews conducted with relevant patients. The measurement model is the needs-based model of quality of life which focuses on the extent to which the disease prevents the fulfilment of human needs. In this respect, the scale is different from, and complementary to, standard measures of health-related quality of life, that assess symptoms and functional limitations. The PlexiQoL is a holistic measure that provides an index of quality of life. The PlexiQoL consists of 22 dichotomous statements, with respondents asked to indicate whether they apply to them 'at the present time'. It can be scored simply by adding the number of positive responses. However, as the scale fits the Rasch measurement model, a more accurate and responsive score can be obtained by producing Rasch transformed scores. The PlexiQoL has been shown to be well accepted by respondents and to have high levels of reproducibility and construct validity.

Pain Measures

1. Pain Intensity

The Numerical Rating Scale-11 (NRS-11) is a self-report segmented 11-point numeric scale that assesses pain intensity. It consists of a horizontal line with 0 representing "no pain" at the right end of the line and 10 representing "worst pain you can imagine" at the left end. Subjects are asked to circle the one number from 0 to 10 that best describes 1) their "most important tumor pain," 2) their "target tumor pain" at its worst during the past week, and 3) their "other pains" (non-tumor) at their worst over the past week. It takes just a few minutes to complete. The NRS-11 is recommended as a core outcome measure of pain intensity for clinical trials.

2. Pain Interference

The Brief Pain Inventory (BPI)—Pain Interference Scale is a 7-item self-report questionnaire that measures the extent to which tumor pain interferes with daily functioning. Subjects are asked to indicate how much tumor pain interfered with various activities (general activity, mood, walking, normal work, relations with other people, sleep, and enjoyment of life) in the past week, with scores ranging from 0 (does not interfere) to 10 (completely interferes). A total score is obtained by taking the mean of the scores for all 7 items; thus, the total pain interference score can range from 0 to 7. This scale takes just a few minutes to complete. These items are recommended to assess pain interference in clinical trials.

Global Impression of Change From

The patient Global Impression of Change (GIC) Scale is a scale that evaluates the clinical significance of changes in pain intensity or other morbidities. We will administer an adapted version of the GIC Scale at the **follow-up** PRO evaluations only (it should not be given pre-study because it assesses change from baseline). On the adapted GIC, patients will give their overall impression of change in their 1) tumor pain, 2) other pain, and 3) tumor-related morbidities from before initiation of the MEK inhibitor to the current evaluation point. The GIC Scale has been used in several research studies on chronic pain in adults. It has been recommended by the

Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) for clinical trials involving chronic pain (Dworkin et al., 2005). The relationship between the numerical scale (NRS-11) and the qualitative scale (GIC) will be determined by breaking the participants into groups based on their qualitative response and determining the mean point difference associated with each qualitative label. This information will help evaluate the effects of the study drug on pain and other morbidities as well as provide data on clinically meaningful change in these problems.

Background Information Form
To allow for more meaningful analysis of the QOL data, the participant (for subjects ≥ 18 years) will complete a background information form at study entry and each subsequent QOL evaluation. This form will collect general information such as the subject's level of education, work status, psychiatric diagnoses, and pain medications, as well as the perceived visibility of NF1 tumors and severity of NF1 symptoms.

Administration Schedule of the PRO Measures
• Baseline and follow-up PRO assessments: Adult participants will complete the PRO measures (except for the PlexiQoL) at baseline (prior to starting treatment) and then again after course 4 ±2 weeks, 8 ±2 weeks, 12 ±2 weeks, 18 ±2 weeks, and 24 ±2 weeks. In addition, they should be administered at the end of treatment ±2 weeks if this is not encompassed in the times above. The PlexiQoL will be completed at baseline (prior to starting treatment) and then again after course 12 ±2 weeks or at the end of treatment ±2 weeks if this occurs prior to the end of course 12.

Specific Administration Guidelines

• Prior to seeing the patients for the **baseline** evaluation, find out the area of the body where the target tumor is located (from the designated investigator at your site) so that you can tell patients this location and write it in the blank space for question #2 on the NRS-11.

• When administering these questionnaires, first review the instructions on each form with the subjects. Mention that you can read or explain any of the items if they need any help reading or if they have any questions.

• For participants who may have difficulty reading, it is permissible to read the items to them, or explain any items they have difficulty understanding, but the patients should answer the items themselves. One way to do this is to give the patient their own form on which to mark his/her answers while the staff person reads the items from another copy of the questionnaire.

• The PlexiQoL is to be administered after the other PRO forms.

• Immediately after the participants are finished filling out the questionnaires and before they leave the clinic, please check each form to make sure that the items are completed correctly (e.g., only one response circled) and that all the applicable items are answered.

--If more than one answer is circled for an item, please have the individual choose one response.

--If they forgot to answer an item, ask them to please try to answer it.

• Prior to seeing patients for all of the **follow-up** evaluations, refer back to the completed baseline NRS-11 pain intensity questionnaire to identify the location of the tumor that the

participant previously specified as their most important tumor and then write this information on the blank line in question #1 on the current form. Also, write in the target tumor rated at baseline for item #2. Specifying these locations will help ensure that the patients rate the severity of pain for the **same tumor across all evaluations**.

Table 2. PROs administered to all adult patients (ages ≥ 18 years)* by age and respondent

Adult Patient Questionnaires	**Age of Patient (years)**
	≥ 18
Background Information	
Self-report: Adult Background Form	√
Pain Intensity	
Self-report: Numeric Rating Scale-11	√
Pain Interference	
Self-report: Brief Pain Inventory	√
NF1 QOL	
Self-report: PedsQL Adult NF1 Disease-specific QOL PlexiQoL	√ √
Global Impression of Change (Follow-up only)	
Self-report: Global Impression of Change Scale	√ (Follow-up evaluations only)

√ Administer items with a check mark under the correct age range of the patient

* IMPORTANT ADMINISTRATION GUIDELINES: If a participant is 18 years or older at study entry, they should be administered the PROs in the Adult Module. However, if a participant is 17 years at study entry they should be administered the PROs in the Pediatric Module, continuing on the pediatric measures even if they turn 18 years old

APPENDIX IX: PLEXIFORM NEUROFIBROMA SYMPTOM CHECKLIST

To be completed by patient if able, otherwise completed by the parent/guardian. To be completed by the same individual at each time point.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

For each item below, please check one box (unless otherwise indicated) to describe how much each of these has been a problem for you in the past two weeks:

1) Fatigue/feeling tired	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
2) Sleep problems	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
3) Less appetite (eating less)	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
4) More appetite (eating more)	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
5) Headaches	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
6) Vision changes	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
7) Decreased hearing	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
8) Mouth Sores	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
9) Trouble swallowing	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
10) Choking	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
11) Snoring	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
12) Frequent awakenings at night	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot

13) Cough	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
14) Wheezing	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
15) Difficulty breathing	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
16) Chest pain	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
17) Palpitations/fluttering	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
18) Shortness of breath with activity	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
19) Shortness of breath at rest	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
20) Swelling in hands/feet	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
21) Abdominal pain	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
22) Heartburn	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
23) Nausea	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
24) Vomiting	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
25) Diarrhea	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
26) Constipation	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
27) Stool incontinence	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
28) Pain with urination	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
29) Increased urinary frequency/urgency	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
30) Difficulty beginning urination	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
31) Urinary incontinence	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
32) Weakness	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot

If yes, where?					
33) Muscle pain	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
If yes, where?					
34) Dizziness	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
35) Numbness	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot
36) Tingling	☐ Not at all	☐ A little	☐ Some	☐ Pretty much	☐ A lot

COMPLETED BY: _____ DATE _____

APPENDIX X: PN LOCATION AND ASSOCIATED MORBIDITIES

INSTRUCTIONS:

To be completed by the Site PI prior to starting treatment

- Assess PN location(s) by clinical exam and imaging studies (target PN and up to 2 non target PN; fill out a separate form for each PN)
- List morbidities present using numbers below (see list)
- If no morbidity present, list potential morbidities based on PN site(s)

PN Location				**PN Associated Morbidities**		
	R	L	B/L	Pain (Y/N)	Present PN Morbidities (using numbers listed below)/ Comments	Potential Morbidities
Orbit						
Face						
Ear canal						
Tongue						
Anterior neck/upper airway						
Posterior neck (cervical paraspinal)						
Mediastinum						
Intra-thoracic						
Thoracic/paraspinal/chest wall						
Liver						
Abdominal						
Mesentery						
Posterior abdomen/pelvis (Lumbo-sacral plexus)						

Perineum/peri-rectal/bladder						
Upper arm (Brachial plexus)						
Forearm						
Hand						
Thigh/upper leg						
Lower leg						
Foot						
Other (please specify):						

Morbidities:			
1- Vision loss 2- Facial motor dysfunction 3- Auditory loss	4- Difficulty swallowing 5- Abnormal speech 6- Airway obstruction 7- Respiratory compromise	8- Bladder dysfunction 9- Bowel dysfunction 10- Motor weakness 11- Decreased range of motion	12- Sensory deficit 13- PN related Disfigurement/ appearance
14- Other (please describe):			

*****Instruction to site PI: Please place asterix next to target lesion morbidity.**

APPENDIX XI: PLEXIFORM NEUROFIBROMA CLINICIAN MORBIDITY ASSESSMENT

Instructions

- To be completed by the treating physician.
- At baseline, please identify morbidities.
- Please fill out a separate form for each PN identified in Appendix X)
- At follow-up, for each morbidity identified at baseline, please document whether the noted morbidity is:
    - worse
    - stable
    - improved but still present
    - resolved
- Please include comments (ex. previously was using 2 pain medications and now using one; previously on Bipap setting of 8, now on Bipap setting of 4). If available, please provide any objective applicable testing results related to morbidities (in the comments box).

Please document the baseline morbidities.

Baseline Morbidity 1: _____

Baseline Morbidity 2: _____

Baseline Morbidity 3: _____

Baseline Morbidity 4: _____

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

For each baseline morbidity, please document whether the morbidity is:
1) worse; 2) stable; 3) improved but still present; or 4) resolved

Evaluation Date	Course	Baseline Morbidity#1	Baseline Morbidity #2	Baseline Morbidity #3	Baseline Morbidity #4	Comments

COMPLETED BY: _____ DATE:_____

APPENDIX XII: FUNCTIONAL OPHTHALMOLOGY EVALUATION FOR ORBITAL PN

Please perform, if feasible, eye exam as outlined below. Additional evaluations can be performed at discretion of the site PI.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

Location of orbital PN? ☐ right ☐ left ☐ both

Does patient have an optic pathway glioma? ☐ Yes ☐ No

Examination Protocol

1. Distance Acuity:
   Testing should be performed using HOTV or Teller Acuity Cards as outlined below. Each eye should be tested separately with best correction in place. The same testing method must be used for each subject throughout their time on study. HOTV should be used for children old enough to perform reliably at the baseline pre-treatment visit. All other children should be tested using Teller Acuity Cards. For analysis, acuity will be corrected for normal at the age of testing by calculating an age-based acuity. Changes $\geq$ 0.2 logMAR will be considered significant.

   HOTV Testing
   HOTV testing should be performed using the HOTV PEDIG ATS protocol at 3 meters. Acuity should be reported in logMAR.

   Teller Acuity Card Testing
   Teller Grating Acuity should be measures at 55 cm and reported in Courses/cm (choose the best card achieved). Grating acuity will be converted to logMAR by the study team.

2. Exophthalmometry
   Measurements should be made with the same equipment for each subject throughout their time on study. Measurements will be made three times during each visit and averaged. The type of exophthalmometer and the base between the orbital rims should be the same during each measurement and each visit unless the child has significant facial growth between visits. Measurement of the amount of proptosis will be reported in mm. For analysis, changes in proptosis > 2 mm will be considered significant.

		OD	OS
Distance Acuity (best corrected):	Teller Acuity (cycle/cm)		
	HOTV (logMAR)		
	If acuity has declined, what is it due to:	☐ Progressive Orbital PN ☐ Progressive amblyopia (deprivation, anisometropic, stabismic, etc) ☐ Optic pathway glioma ☐ Other (describe):	☐ Progressive Orbital PN ☐ Progressive amblyopia (deprivation, anisometropic, stabismic, etc) ☐ Optic pathway glioma ☐ Other (describe):
Exophthalmometry (mm)	Measurement #1		
	Measurement #2		
	Measurement #3		
	Average		

COMPLETED BY: _____ **DATE:** _____

APPENDIX XIII: MOTOR/STRENGTH EVALUATIONS

Endurance Evaluation

All patients ≥ 5 years at enrollment and ambulatory, with lower extremity PN, cord compression, or airway PN, should undergo endurance testing using the 6-Minute Walk Test. This evaluation should be performed by Rehabilitation Medicine, Physiatry, or Physical Therapy, if possible.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

Height (cm):_____ Weight (kg):_____
Gender:_____ Age (year and months): _____

TOTAL DISTANCE (METERS)	
HEART RATE	
Before Walk	
At Completion of Walk	
After 5 Minute Cool-down	
BLOOD PRESSURE	
Before Walk	
After Completion of Walk	
After 5 Minute Cool-down	
VELOCITY (Total Distance (m)/6 Minutes)	

Percentage of normal* distance achieved: _____

* Normal values adjusted for age, BMI, and sex as per: Geiger et al. Six-Minute walk test in children and adolescents. J of Pediatrics, 2007

COMPLETED BY: _____ DATE: _____

Strength Evaluation

Baseline evaluation:

All patients with PN that cause or have the potential to cause motor dysfunction, weakness, or cord compression should undergo evaluation of strength of all muscle groups at baseline. This evaluation should be performed by Rehabilitation Medicine, Physiatry, or Physical Therapy, if possible, and should ideally be completed by the same examiner at each visit, if feasible.

Please grade strength in the relevant muscle groups using Medical Research Council (MRC) grading of 0-5/5 (+/- may be used). Please grade grip and pinch strength in pounds of force. *(Medical Research Council. Aids to the examination of the peripheral nervous system, Memorandum no. 45, Her Majesty's Stationery Office, London, 1981)*

Follow-up evaluations:

At follow-up exams, a focused evaluation of strength of the affected and potentially affected muscle groups should be performed. The contralateral side should be tested for comparison. If the contralateral side is also potentially affected, a third extremity should be tested as an assessment of overall strength. The muscle group(s) to be assessed at follow-up evaluations will be specified by the study team based on PN location and baseline findings and will be indicated below:

☐ Upper Extremity ☐ Lower Extremity ☐ Other (Please specify):

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

Manual Muscle/Strength Testing (include affected and at-risk muscle groups)

POSITION		LEFT	RIGHT	MIDLINE
SUPINE	Neck Flexors			
SIDELYING	Gluteus Medius			
PRONE	Gluteus Maximus			
	Hamstrings			
	Plantar flexors			
	Neck extensors			
SITTING	Trapezius			
	Deltoids			
	Biceps			
	Triceps			
	Wrist flexors			
	Wrist extensors			
	Iliopsoas			
	Quadriceps			
	Anterior tibial			
	Gastrocnemius			
	Extensor Hallucis Longus			

POSITION	LEFT	RIGHT
Grip*		
Key Pinch*		

*Please grade grip and pinch strength in pounds of force

COMPLETED BY: _____ **DATE:** _____

Range of Motion Evaluation

Baseline evaluation:
All patients with PN that cause or have the potential to cause motor dysfunction, weakness, or cord compression should undergo evaluation of range of motion of all joints at baseline. This evaluation should be performed by Rehabilitation Medicine, Physiatry, or Physical Therapy, and should ideally be completed by the same examiner at each visit, if feasible.

Follow-up evaluations:
At follow-up exams, a focused evaluation of range of motion of the affected and potentially affected joints should be performed. The contralateral side should be tested for comparison. Joint(s) to be assessed at follow-up evaluations will be specified by the study team based on PN location and baseline findings and indicated below:

☐ Neck ☐ Upper Extremity ☐ Lower Extremity

☐ Other (Please specify):

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

LEFT (degrees)		RIGHT (degrees)	MIDLINE (degrees)
	NECK		
	Extension (Sidelying)		
	Flexion (Sidelying)		
	Lateral Flexion (Supine)		
	Rotation (Supine)		
	UPPER LIMB		
	SHOULDER (Supine)		
	Abduction		
	Internal Rotation		
	External Rotation		
	ELBOW (Supine)		
	Flexion		
	Extension		
	WRIST (Supine)		
	Flexion		
	Extension		
	LOWER LIMB		
	HIP		
	Flexion (Supine)		
	Extension (Sidelying)		
	External Rotation (Supine)		
	Internal Rotation (Supine)		
	Abduction (Supine)		
	KNEE (Supine)		
	Flexion		
	Extension		
	ANKLE (Supine)		
	Plantarflexion		
	Dorsiflexion		

COMPLETED BY: _____ **DATE:** _____

Leg Length Evaluation

All patients with PN located in the lumbosacral plexus or below (i.e. lower limb) should undergo leg length measurements to evaluate for discrepancy. This evaluation should be performed by Rehabilitation Medicine, Physiatry, or Physical Therapy, and should ideally be completed by the same examiner at each visit, if feasible. Measurements should be taken from the Anterior Superior Iliac Spine to the Medial Malleolus of the ankle. The subject should be lying supine with hips and knees extended.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

LEG LENGTH (cm)

LEFT	RIGHT

Leg length discrepancy (difference between right and left): _____

If leg length discrepancy present, is there associated pelvic obliquity: ☐ Yes ☐ No

COMPLETED BY: _____ DATE: _____

Grooved Pegboard Test

All patients ≥ 5 years at enrollment and ability to use one or both upper extremities with PN located in the upper extremities or patients with known cervical or upper thoracic cord compression should undergo Grooved Pegboard Testing.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

Age: _____ Gender: _____

GROOVED PEGBOARD SCORE SHEET

Dominant Hand (Circle one): LEFT RIGHT

	Time (Sec)	# of drops	# of pegs placed	X ± SD*	Z*
Dominant Hand					
Non-Dominant Hand					

*To be completed by the operations center

COMPLETED BY: _____ DATE: _____

GROOVED PEGBOARD INSTRUCTIONS

This is a pegboard and these are the pegs. (The examiner points out each and then picks up one of the pegs and continues). **All the pegs are the same, that is, they have a round side and a square side and so do the holes in the board. What you must do is match the groove of the peg with the groove in the board and put these pegs into the holes like this.**

(The examiner demonstrates by filling the top row. Then remove the pegs, putting them back into the tray. For the right-hand trial, the examiner demonstrates that the pegs are placed from the patient's left to right, and from right to left for the left-hand trial. The dominant hand trial is administered first, followed by the non-dominant hand trial).

For adolescents (ages ≥9 to <15) and adults (age ≥ 15 years):

When I say go, begin here and put the pegs into the board as fast as you can, using only your (dominant) **hand. Fill the top row completely from this side to this side** (from left to right for right-handed patients, from right to left for left-handed). **Do not skip any. Fill each row the same way you filled the top row. Any questions? Ready, as fast as you can, go.** (Repeat this task with the non-dominant hand)

For children (ages ≥5 to <9): Use 10 pegs only

When I say go, begin here and put the pegs into the board as fast as you can, using only your (dominant) **hand. Fill the top row completely from this side to this side** (from left to right for right-handed patients, from right to left for left-handed). **Do not skip any. Then go on to the second row and fill it the same way you filled the top row. Stop when you have completed the second row. Any questions? Ready, as fast as you can, go.**
(Repeat this test with the non-dominant hand).

APPENDIX XIV: BLADDER AND BOWEL PATIENT QUESTIONNARIE

Patients with bowel/bladder dysfunction or their guardians should complete the questionnaire below. *Not applicable for subjects that are not toilet trained*.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

To be completed by Patient if able, otherwise completed by the Parent/Guardian:

1. I pee in my underwear during the day:
□ Never □ 1 day a week □ 2–3 days a week □ 4–5 days a week □ Every day

2. When I pee in my underwear, they are:
□ I don't pee in my underwear □ Almost dry □ Damp □ Wet □ Soaked

3. In a normal day I go to the washroom to pee:
□ 1–2 times □ 3–4 times □ 5–6 times □ 7–8 times □ More than 8 times

4. I feel that I have to rush to the washroom to pee:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

5. I hold my pee by crossing my legs or sitting down:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

6. It hurts when I pee:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

7. I wet my bed at night:
□ Never □ 1-2 nights per week □ 3-4 nights per week □ 4–5 nights per week □ Every night

8. I wake up to pee at night:
□ Never □ 1-2 nights per week □ 3-4 nights per week □ 4–5 nights per week □ Every night

9. When I pee, it stops and starts:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

10. I have to push or wait for my pee to start:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

11. I have bowel movements (poop):
□ More than every day □ Every day □ Every other day □ Every 3 days

12. My stool (poop) is hard:
□ Never □ Less than half of the time □ Half of the time
□ More than half of the time □ Every day

13. I have bowel (poop) accidents in my underwear:
□ Never □ 1–2 times per week □ 3 times per week □ 4–5 times per week □ Every day

14. How easy was it to answer these questions?
□ Very easy □ Easy □ Neither easy nor difficult □ Difficult □ Very difficult

COMPLETED BY: _____ **DATE:** _____

APPENDIX XV: FUNCTIONAL HEARING EVALUATION FOR PN THAT COMPRESS THE EAR CANAL, CAUSE HEARING LOSS, OR POTENTIALLY CAN CAUSE HEARING LOSS

Please perform, if feasible, audiogram OR auditory brainstem response (ABR/BAER), and a word recognition score *as clinically indicated and per institutional practice*. Additional evaluations can be performed at the discretion of the site PI.

Testing should be performed pre-treatment, and then post to courses 4, 8, 12, 18, 24.

Select one:

Pre-treatment ☐ Post course 4 ☐ Post course 8 ☐ Post course 12 ☐

Post course 18 ☐ Post course 24 ☐

Other (Please specify): _____

Location of PN affecting or potentially affecting? ☐ right ☐ left ☐ both

Hearing Test Results (measured in dB HL)
Please provide results for all frequencies (Hz) tested
NT = not tested

Air Conduction Thresholds

	250 Hz	500	1000	2000	3000	4000	6000	8000
Right								
Left								

Bone Conduction Thresholds

	250 Hz	500	1000	2000	3000	4000	6000	8000
Right								
Left								
Unmasked								

ABR/BAER Results (measured in dB eHL)
Please provide results for all frequencies (Hz) tested
NR = No response
NT = not tested

Air Conduction Thresholds

	500 Hz	1000	2000	4000	8000
Right					
Left					

Bone Conduction Thresholds

	500 Hz	1000	2000	4000	8000
Right					
Left					

Word Recognition Score

Please report % words correctly recognized at the optimal dB level based on the speech awareness threshold / speech recognition threshold clinically used

	%
Right	
Left	

APPENDIX XVI: PHOTOGRAPHY OF VISIBLE PN

Photography of visible PN (include visible target and any non-target PN that was identified in Appendix X) will be performed in consenting patients. Please store the photos on a password protected computer. Please follow the study memos from the operations team for future instructions regarding photograph submission.

Testing should be performed pre-treatment, and then post courses 4, 8, 12, 18, 24.

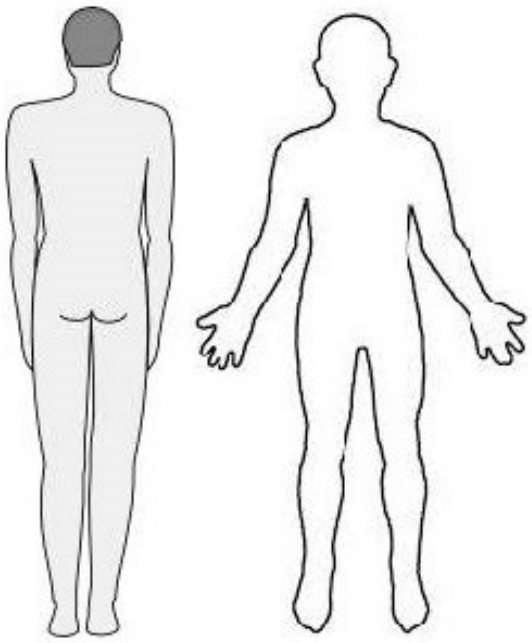
All photographs at each time point should be taken from the same distance, using similar lighting and camera settings, if possible. Baseline photographs should be present at the time of follow up photography to allow for the best possible comparison.

Please fill out this form at baseline to indicate which body regions are being photographed at the specified time points.

Please Circle and Describe the location of each PN being photographed:

Description of Photo body location:

#1:



#2:

#3:

#4:

#5:

COMPLETED BY: _____ DATE: _____

APPENDIX XVII: BLOOD PRESSURE NORMS FOR SUBJECTS < 18 YEARS OF AGE

In order to use the tables below, please note that the height percentile is determined by the standard growth charts. The child's measured systolic and diastolic BP is compared with the numbers provided in the table (boys or girls) for age and height percentile. The child is normotensive if BP is below the 90th percentile. If the child's BP (systolic or diastolic) is at or above the 95th percentile, the child may be hypertensive and repeated measurements are indicated. BP measurements between the 90th and 95th percentiles are high-normal and warrant further observation and consideration of other risk factors.

90th and 95th Percentile BP Girls

Age (y)	Blood Pressure Percentile	Systolic Blood Pressure by Percentile of Height (mm Hg)							Diastolic Blood Pressure by Percentile of Height (mm Hg)						
		5%	10%	25%	50%	75%	90%	95%	5%	10%	25%	50%	75%	90%	95%
1	90th	94	95	97	98	100	102	102	50	51	52	53	54	54	55
	95th	98	99	101	102	104	106	106	55	55	56	57	58	59	59
2	90th	98	99	100	102	104	105	106	55	55	56	57	58	59	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
3	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	111	112	113	63	63	64	65	66	67	67
4	90th	102	103	105	107	109	110	111	62	62	63	64	65	66	66
	95th	106	107	109	111	113	114	115	66	67	67	68	69	70	71
5	90th	104	105	106	108	110	112	112	65	65	66	67	68	69	69
	95th	108	109	110	112	114	115	116	69	70	70	71	72	73	74
6	90th	105	106	108	110	111	113	114	67	68	69	70	70	71	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
7	90th	106	107	109	111	113	114	115	69	70	71	72	72	73	74
	95th	110	111	113	115	116	118	119	74	74	75	76	77	78	78
8	90th	107	108	110	112	114	115	116	71	71	72	73	74	75	75
	95th	111	112	114	116	118	119	120	75	76	76	77	78	79	80
9	90th	109	110	112	113	115	117	117	72	73	73	74	75	76	77
	95th	113	114	116	117	119	121	121	76	77	78	79	80	80	81
10	90th	110	112	113	115	117	118	119	73	74	74	75	76	77	78
	95th	114	115	117	119	121	122	123	77	78	79	80	80	81	82
11	90th	112	113	115	117	119	120	121	74	74	75	76	77	78	78
	95th	116	117	119	121	123	124	125	78	79	79	80	81	82	83
12	90th	115	116	117	119	121	123	123	75	75	76	77	78	78	79
	95th	119	120	121	123	125	126	127	79	79	80	81	82	83	83
13	90th	117	118	120	122	124	125	126	75	76	76	77	78	79	80
	95th	121	122	124	126	128	129	130	79	80	81	82	83	83	84
14	90th	120	121	123	125	126	128	128	76	76	77	78	79	80	80
	95th	124	125	127	128	130	132	132	80	81	81	82	83	84	85
15	90th	123	124	125	127	129	131	131	77	77	78	79	80	81	81
	95th	127	128	129	131	133	134	135	81	82	83	83	84	85	86
16	90th	125	126	128	130	132	133	134	79	79	80	81	82	82	83
	95th	129	130	132	134	136	137	138	83	83	84	85	86	87	87
17	90th	128	129	131	133	134	136	136	81	81	82	83	84	85	85
	95th	132	133	135	136	138	140	140	85	85	86	87	88	89	89

90th and 95th Percentile Boys

Age (y)	Blood Pressure Percentile	Systolic Blood Pressure by Percentile of Height (mm Hg)							Diastolic Blood Pressure by Percentile of Height (mm Hg)						
		5%	*10%*	*25%*	*50%*	*75%*	*90%*	*95%*	*5%*	*10%*	*25%*	*50%*	*75%*	*90%*	*95%*
1	90th	94	95	97	98	100	102	102	50	51	52	53	54	54	55
	95th	98	99	101	102	104	106	106	55	55	56	57	58	59	59
2	90th	98	99	100	102	104	105	106	55	55	56	57	58	59	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
3	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	111	112	113	63	63	64	65	66	67	67
4	90th	102	103	105	107	109	110	111	62	62	63	64	65	66	66
	95th	106	107	109	111	113	114	115	66	67	67	68	69	70	71
5	90th	104	105	106	108	110	112	112	65	65	66	67	68	69	69
	95th	108	109	110	112	114	115	116	69	70	70	71	72	73	74
6	90th	105	106	108	110	111	113	114	67	68	69	70	70	71	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
7	90th	106	107	109	111	113	114	115	69	70	71	72	72	73	74
	95th	110	111	113	115	116	118	119	74	74	75	76	77	78	78
8	90th	107	108	110	112	114	115	116	71	71	72	73	74	75	75
	95th	111	112	114	116	118	119	120	75	76	76	77	78	79	80
9	90th	109	110	112	113	115	117	117	72	73	73	74	75	76	77
	95th	113	114	116	117	119	121	121	76	77	78	79	80	80	81
10	90th	110	112	113	115	117	118	119	73	74	74	75	76	77	78
	95th	114	115	117	119	121	122	123	77	78	79	80	80	81	82
11	90th	112	113	115	117	119	120	121	74	74	75	76	77	78	78
	95th	116	117	119	121	123	124	125	78	79	79	80	81	82	83
12	90th	115	116	117	119	121	123	123	75	75	76	77	78	78	79
	95th	119	120	121	123	125	126	127	79	79	80	81	82	83	83
13	90th	117	118	120	122	124	125	126	75	76	76	77	78	79	80
	95th	121	122	124	126	128	129	130	79	80	81	82	83	83	84
14	90th	120	121	123	125	126	128	128	76	76	77	78	79	80	80
	95th	124	125	127	128	130	132	132	80	81	81	82	83	84	85
15	90th	123	124	125	127	129	131	131	77	77	78	79	80	81	81
	95th	127	128	129	131	133	134	135	81	82	83	83	84	85	86
16	90th	125	126	128	130	132	133	134	79	79	80	81	82	82	83
	95th	129	130	132	134	136	137	138	83	83	84	85	86	87	87
17	90th	128	129	131	133	134	136	136	81	81	82	83	84	85	85
	95th	132	133	135	136	138	140	140	85	85	86	87	88	89	89

APPENDIX XVIII: INSTRUCTIONS FOR PREPARATION OF MEK162 (BINIMETINIB) ORAL SUSPENSION

Objective

This document describes in detail the preparation procedure of MEK162 (binimetinib) oral suspensions from MEK162 15 mg film-coated tablet (FCT) and Ora Sweet'/ water 3:1 (v/v) vehicle. This document also provides the instruction for administration of the reconstituted oral suspensions to the subjects.
Safety instructions

Utmost care must be exercised while handling (preparation and dosing) MEK162 oral suspensions. Follow the hazardous waste disposal procedures applicable to the clinic's site for the disposal of contaminated material (e.g. gloves, paper towels, syringes etc.). Avoid direct contact with clothes and skin.

In the event of spillage of a MEK162 suspension on hard washable surface. e.g.. work bench. table or floor:

- Cleaning should be done while wearing protective equipment (minimum requirement: disposable gloves).
- Wipe the area with clean paper towel until the area is dry.
- Use fresh, clean paper towel for each wiping action.
- Wipe the area twice with Ethanol 70% (wear chemically resistant gloves).
- Wipe the area with clean water and dry with a clean paper towel.
- Dispose the wet towels in the appropriate waste bags.

Breakage of a glass bottle containing the MEK162 suspension:

- Do not attempt to pick up broken glass as it can pierce through gloves and might cut the skin.
- Use a brush to pick up the broken glass pieces.
- Wipe the area with a wet paper towel to remove any trace of broken glass.
- Wipe the area with clean paper towel until the area is dry.
- Wipe the area twice with Ethanol 70% (wear chemically resistant gloves).
- Wipe the area with clean water and dry with a clean paper towel.
- Dispose the waste in the appropriate waste bags.

In case of contact with clothes or skin, remove contaminated clothing, and rinse contaminated skin immediately with plenty of water and soap.

Materials

- MEK162 15 mg film coated tablet (provided by Array BioPharma, , a wholly owned subsidiary of Pfizer Inc.)
- 125 mL glass bottle or 4 ounce plastic bottle
- Child-resistant screw cap
- Uncarbonated potable water (from any commercial source).
- 30 mL syringe (i.e. 30 ml accuracy ± 0.5 ml).
- Dosing cup adapted to target administration volume (i.e. 15 to 50 ml).
- Exacta-Med syringe type dispenser sets
- Ethanol 70% (disinfectant and cleaning agent; used in event of spillage).

- Plastic bags (large enough to hold one 125 ml brown glass bottle).
- Ora Sweet® vehicle
- Disposable gloves

Preparation of MEK162 oral suspension at 1mg/mL

Note: Preparation of oral solution is to be carried out by the Pharmacy using clean glassware, measuring syringe, etc. Suspension, Exacta-Med syringe type dispenser sets, and dosing cups are provided to the patients after preparation. The suspension must be stored at room temperature (below 25°C) and should not be refrigerated. It should be used within 30 days after preparation.

- Step 1: Measure 15 mL uncarbonated potable water using the 30 mL syringe and transfer it into the 125 mL brown bottle (or 4 oz plastic bottle).
- Step 2: Add four (4) MEK162 15 mg film coated tablets into the 125mL brown bottle (or 4 oz plastic bottle)
- Step 3: Close the bottle using the screw cap and let the bottle sit for 10 minutes.
- Step 4: Shake the bottle for 1-3 minutes until the tablets are completely dispersed. Remove the cap from the bottle.
- Step 5: Measure 45 mL Ora Sweet using a 30 mL syringe and transfer it into the 125 mL brown bottle (or 4 oz plastic bottle).
- Step 6: Shake the bottle for 1-3 minutes. Confirm the suspension is homogenous.

Administration of MEK162 oral suspension at 1mg/mL

Note: Each administration should be performed using a new Exacta-Med syringe type dispenser. If dosing cups are used for administration, a new dosing cup needs to be used for each dispensing. The suspension must be stored at room temperature (below 25°C) and should not be refrigerated. It should be used within 30 days after preparation.

- Remove the screw cap from the brown bottle which contains the oral suspension.
- Plug the 10 mL syringe adapter onto the bottleneck and close the brown bottle with the screw cap.
- Gently handshake the bottle of oral suspension for 60 seconds just prior to dosing to make sure the content is homogeneous.
- Remove the screw cap from the brown bottle and connect the syringe tip to the adapter with the bottle in upright position.
- Hold the adapter and bottle in upside-down position, and carefully draw the required volume of oral suspension using the 10 mL syringe. After this is done, hold the bottle in upright position and remove the filled syringe from the adapter.
- Transfer the content directly from the syringe into the mouth of the patient. Use a new syringe for each administration if more than 10mL of suspension is needed (discard the syringe after every use).

If more than 10mL of suspension is needed, the content from the syringes can alternatively be transferred into a dosing cup, and administered to the patient from the cup. In this case, care has

to be taken to ensure all the suspension contained in the cup is administered. Use a new dosing cup for each administration.

- Close the brown bottle with the cap immediately after use.
- The Exacta-Med syringe type dispenser set and the dosing cup should be discarded after each administration.
- The dosing bottle containing the remainder of the prepared suspension can be stored at room temperature, below 25°C, and should not be refrigerated. It should be discarded after 30 days.
- The dosing bottle with any unused suspension should be returned to the pharmacy.

Storage instructions

The reconstituted suspension can be stored at room temperature no greater than 25°C and should not be refrigerated. It must be used within 30 days after preparation.

APPENDIX XIX: PEDIATRIC DOSAGE OF BINIMETINIB (MEK162) FOR LIQUID SUSPENSION

BSA (m^2)	Level 0 ($32mg/m^2$)	Level -1 ($24mg/m^2$)	Level -2 ($16mg/m^2$)	Level -3 ($12mg/m^2$)
≤0.14	3	2	2	1
0.15-0.24	6	5	3	2
0.25-0.34	10	7	5	4
0.35-0.44	13	10	6	5
0.45-0.54	16	12	8	6
0.55-0.64	19	14	10	7
0.65-0.74	22	17	11	8
0.75-0-.84	26	19	13	10
0.85-0.94	29	22	14	11
0.95-1.04	32	24	16	12
1.05-1.14	35	26	18	13
1.15-1.24	38	29	19	14
1.25-1.34	42	30	21	16
≥1.35	45	30	22	17

BSA should be rounded to the nearest tenth. Quantities listed are individual doses in mg amounts. Dose should be given twice daily (every 12 hours)

APPENDIX XX: PEDIATRIC DOSAGE OF BINIMETINIB (MEK162) FOR TABLETS

Dosing table (15 mg tablets)

BSA (m^2)	Level 0 (32mg/m^2)	Level -1 (24mg/m^2)	Level -2 (16mg/m^2)	Level -3 (12mg/m^2)
≤0.42	*	*	*	*
0.43-48	15 mg (1 tablet)	*	*	*
0.49-0.55	15 mg (1 tablet)	*	*	*
0.55-0.63	*	15 mg (1 tablet)	*	*
0.64-0.67	*	15 mg (1 tablet)	*	*
0.68-0.72	*	15 mg (1 tablet)	*	*
0.73-0.84	*	*	*	*
0.85-0.97	30 mg (2 tablets)	*	15 mg (1 tablet)	*
0.98-1.06	30 mg (2 tablets)	*	15 mg (1 tablet)	*
1.07-1.12	30 mg (2 tablets)	*	*	*
1.13-1.24	*	30 mg (2 tablets)	*	15 mg (1 tablet)
≥1.25	45 mg (3 tablets)	30 mg (2 tablets)	*	15 mg (1 tablet)

*It is not permitted to use tablet form only at this BSA for this dose level. See suspension dosing in Appendix XIX. Suspension OR a combination of tablets and liquid may be used to achieve the dose. For example, if the dose were 22 mg, and the volume of liquid was too challenging for the patient to take, they may take a 15 mg tablet + 7 mg liquid to achieve the dose. Dose should be given twice daily (every 12 hours).

1) Blood

Prior to the day of blood draw, the site will have arranged for a sample collection kit supplied by the biorepository to be shipped to the site. The Center for Data Driven Discovery in Biomedicine (D3b) at CHOP will be providing kits and labels. The kit will contain tubes and bar-coded labels (which include a specimen number) for blood collection. It will also contain shipping materials and instructions for sending collected blood samples to the biorepository, as well as a pre-paid shipping label.

Samples

- Streck tube (for plasma): ~ 2 mL.
- EDTA tube (for DNA): ~ 5 mL.
- PAXGene tube (for RNA) ~ 2.5 mL.

Tubes should be inverted at least 10 times after being drawn.

The samples will only be obtained if the total blood volume does not exceed 5 mL/kg. If not enough blood can be drawn for all tubes, the priority order for obtaining the specimens is: 1) Streck tube, 2) EDTA tube, 3) PAXGene tube.

Label the specimens with the specimen labels provided with the specimen kit.

Blood samples must be shipped (unprocessed) same day at ambient temperature to the biorepository by overnight delivery using shipping container provided by the biorepository. Samples must be shipped Monday through Thursday.

Enter the specimen numbers into the NF Consortium Operations Center eDES.

For questions about shipping, kits, tubes, etc., and to confirm the shipping address prior to sending samples, please contact: Elizabeth Appert (apperte@email.chop.edu) and BioRc (biorc@email.chop.edu).

2) Tumor Tissue

Prior to the day of surgery, the site will have arranged for a tissue sample collection kit supplied by the biorepository to be shipped to the site. The Center for Data Driven Discovery in Biomedicine (D3b) at CHOP will be providing kits and labels. The kit will contain bar-coded cryovials (which include a specimen number) for tissue collection. It will also contain shipping materials and instructions for sending collected tissue samples to the biorepository, as well as a pre-paid shipping label.

Samples

- Fresh frozen tissue: 1-2 pea sized aliquots (~ 0.3 cm^3, equivalent to 0.8 cm in diameter) of excess (left over) tumor tissue. Place each aliquot in a separate cryovial and label the specimen with subject's study number. Snap freeze the aliquots in liquid nitrogen (preferred) or dry ice and then store in a freezer at -80°C or below until they can be sent to the biorepository.
- 1 H&E-stained slide cut from each submitted piece to confirm tumor is present.
- If fresh frozen tissue is unavailable, please submit processed DNA (at least 3 micrograms) and RNA (at least 400 nanograms with optimal RIN > 7) if available.

Label the specimen manifest with the subject's study number.
Fresh frozen tissue or DNA/RNA must be shipped on dry ice via overnight delivery using shipping materials provided by the biorepository. The H&E slide should be shipped at ambient temperature. Samples must be shipped Monday through Thursday.

Enter the specimen numbers into the NF Consortium Operations Center eDES.

For questions about shipping, kits, tubes, etc., and to confirm the shipping address prior to sending samples, please contact: Elizabeth Appert (apperte@email.chop.edu) and BioRc (biorc@email.chop.edu).