CLINICAL STUDY REVISED PROTOCOL

A Randomized, Double-Blind, Vehicle-Controlled, Parallel Group Phase 2a Study to Determine Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Activity of NFX-179 Gel in Subjects with Cutaneous Neurofibromas

Protocol No.	NFX-179-NF1-201
Revised Protocol Date:	24-JUL-2020
Revised Protocol No.:	1 (incorporates Amendment Number 1)
Supersedes:	N/A
Sponsor:	NFlection Therapeutics 714 Woodcrest Road, Wayne, PA 19087 United States
Medical Monitor:	Guy Webster, M.D. Ph.D. 24-hour telephone: (302) 559-8684 Email: GWebster@Nflectionrx.com

REVISED PROTOCOL INVESTIGATOR SIGNATURE PAGE

Number: NFX-179-NF1-201

Revised Protocol Number 1 (incorporates Amendment Number 1)

INVESTIGATOR COMMITMENT:

I will provide copies of the protocol, any subsequent protocol amendments and access to all information provided by NFlection to the investigational center staff under my supervision. I will discuss this material with them to ensure that they are fully informed about the Investigational Medicinal Product and the study protocol.

I agree to conduct this clinical study according to the attached protocol, except when mutually agreed to with NFlection in writing. I also agree to conduct this study in compliance with all local regulatory requirements, Good Clinical Practices, as well as with the requirements of the appropriate Institutional Review Board(s) /Ethics Committee(s) and any other Institutional requirements.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

REVISED PROTOCOL APPROVAL PAGE

Protocol Number: NFX-179-NF1-201
Revised Protocol Number: 1

A Randomized, Double-Blind, Vehicle-Controlled, Parallel Group Phase 2a Study to Determine Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Activity of NFX-179 Gel in Subjects with Cutaneous Neurofibromas

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Sponsor:	NFlection Therapeutics 714 Woodcrest Road, Wayne, PA 19087 United States

C. Cole 07-27-2020

Christopher Powala
Chief Executive Officer
NFlection Therapeutics

Date

7/27/20

Guy Webster, M.D., Ph.D.
Chief Medical Officer
NFlection Therapeutics

Date

SYNOPSIS

Name of Sponsor:	NFlection Therapeutics, Inc.
Name of Investigational Product:	NFX-179 Gel for topical application
Name of Active Ingredient:	NFX-179
Title of Study:	A Randomized, Double-Blind, Vehicle-Controlled, Parallel Group Phase 2a Study to Determine Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Activity of NFX-179 Gel in Subjects with Cutaneous Neurofibromas
Phase of Development:	2a
Investigational Centers:	Approximately 6 United States investigational centers
Study Period:	Approximately 84 days
Duration of Treatment:	28 days of once-daily (QD) application to 5 Target cNF Tumors
Duration of Subject Participation:	Approximately 84 days: • Screening period: Up to 28 days • Treatment period: 28 days • Follow-up period: 28 days
Objectives:	Primary Objectives: • To determine the safety and tolerability of treatment with NFX-179 Gel 0.05%, 0.15% and 0.50% when applied QD for 28 day • To determine the pharmacodynamic activity of NFX-179 Gel as defined by suppression of phospho-ERK (p-ERK) levels in defined cutaneous neurofibroma (cNF) tumors for each NFX-179 Gel group compared with the Vehicle Gel group after 28 days of QD treatment. Secondary Objectives: • To determine the effect of NFX-179 Gel defined as the percent change in neurofibroma volume after 28 days of QD treatment based on tumor volume derived from ruler measurements, ultrasound and digital imaging • To determine the effect of NFX-179 Gel on the change from baseline in the Subject's Self-Assessment after 28 days of QD treatment • To determine the effect of NFX-179 Gel on the change from baseline in the Physician's Tumor Assessment after 28 days of QD treatment.

Endpoints:	Primary Endpoints: • Pharmacodynamic activity of NFX-179 Gel as defined by suppression of p-ERK levels in Target cNF Tumors in each NFX-179 Gel group compared with the Vehicle Gel group after 28 days of QD application • Safety and tolerability of treatment with NFX-179 Gel (0.05%, 0.15% and 0.5.%) when applied QD for 28 days • Assessment of adverse events (AEs)/serious adverse events (SAEs). Secondary Endpoints: • Percent change in cNF tumor volume after 28 days of QD application • Systemic exposure of NFX-179 Gel during the clinical study after 28 days of QD application • Change in Physician's Tumor Assessment grade after 28 days of QD application • Change in Subject's Self-Assessment grade after 28 days of QD application.
Number of Subjects:	Approximately 48 subjects (≥ 18 years of age) with cNF tumors will be enrolled and randomized in the study. With a 1:1:1:1 randomization ratio, it is anticipated there will be: • 12 subjects randomized to receive NFX-179 Gel 0.05% • 12 subjects randomized to receive NFX-179 Gel 0.15% • 12 subjects randomized to receive NFX-179 Gel 0.50% • 12 subjects randomized to receive Vehicle Gel
Study Design:	This is a 28-day double-blind, randomized, vehicle-controlled, parallel-group phase 2a study to determine the safety, tolerability, pharmacokinetics, and pharmacodynamic activity of 3 dose levels of NFX-179 Gel (0.05%, 0.15% and 0.5.%) with that of vehicle in subjects with NF1. The study drug will be applied topically QD to the 5 Target cNF Tumors for 28 days. At Baseline, the investigator will select 6 Study cNF Tumors (5 Target cNF Tumors for treatment and 1 Untreated cNF Tumor) that are raised and have a diameter of at least 5mm and no larger than 10 mm and a height (thickness) of ≥2mm. One of the Target cNF Tumors must be on the face. **The Untreated cNF Tumor will NOT receive study medication applications.** On Day 28 all 6 Study cNF Tumors will be excised for measurement of p-ERK levels. The excisions will be done 4 hours (+/- 1 hour) after the final study medication application. From the time of consent and for the duration of the study, subjects will be monitored for safety. The Target cNF Tumors will be evaluated for application site reactions at Visits 2 (Baseline), 3, 4 and 5 (End of Treatment). The Target cNF Tumor dimensions will be measured by the investigator at Visits 1 (screening), 2 and 5.

	Blood samples for complete blood count and serum chemistry and urine for urinalysis will be collected from subjects at Visits 1, 2 and 5. Blood samples for pharmacokinetic assessment of plasma levels of NFX-179 will be collected at Visit 2 (Baseline) prior to the first study medication application and at Visit 5 (End of Treatment).
Main Criteria for Inclusion:	Select Inclusion Criteria: 1. Subject is at least 18 years of age 2. Subject must provide written informed consent prior to any study procedures 3. Subject must have a clinical diagnosis of NF1 4. Subject has 6 Study cNF Tumors (5 Target cNF Tumors [1 on the face; 4 on the anterior trunk or upper extremities] that will be treated with the assigned study medication;1 Untreated cNF Tumor on the anterior trunk or upper extremities) that each meet the following criteria: • Has, in the investigator's opinion, a clinically typical appearance • Is dome shaped • Is not pedunculated • Is a discrete tumor • Is not irritated • Is not in an area subject to repeated trauma (*e.g.*, area that is shaved, on the beltline, under a bra strap, etc.) • Does not have an active cutaneous infection • Has a diameter that is ≥5mm and ≤10mm • Has a height of ≥2mm • Is, when centered in the center of the provided template, the only cNF tumor visible • Is not within 5mm of the orbital rim. 5. Subject is willing to have the 5 Target cNF Tumors and the 1 Untreated cNF Tumor excised at the end of the treatment period 6. Subject is willing to have hair in the area surrounding the Target cNF Tumors shaved, if necessary, to obtain photographs 7. Subject is willing to minimize exposure of each Target cNF to natural and artificial ultraviolet radiation 8. Subject is willing to forego treatment of the Target cNF Tumors, except protocol specified therapy, during the study 9. Female subjects who are women of childbearing potential must have a negative urine pregnancy test result and be willing to use a protocol approved, contraceptive method for the duration of the study 10. Subject is willing and able to follow all study instructions and to attend all study visits.

	Select Exclusion Criteria: 1. Subject has applied any of the following topical products in the previous 30 days on or in proximity to any Study cNF Tumor that, in the investigator's opinion, impairs evaluation of any the tumor or which exposes the subject to an unacceptable risk by study participation: • Corticosteroids • Retinoids (*e.g.*, tazarotene, tretinoin, adapalene) • > 5% of an alpha-hydroxy acid (*e.g.*, glycolic acid, lactic acid) • Fluorouracil • Imiquimod 2. Any Study cNF Tumor has ever been treated with an MEK inhibitor or a BRAF inhibitor 3. The subject has used any of the following systemic medications in the noted time period: • Retinoids (*e.g.*, etretinate, isotretinoin) within the previous 90 days • MEK inhibitors within the previous 180 days • BRAF inhibitors within the previous 180 days 4. Subject has a history of hypersensitivity to any of the ingredients in the study medications 5. Subject has any known intercurrent illness or physical condition that would, in the investigator's opinion, impair evaluation of a Target cNF Tumor or which exposes the subject to an unacceptable risk by study participation 6. Subject has, in the investigator's opinion, clinically relevant history of liver disease, including viral hepatitis, current alcohol abuse, or cirrhosis 7. Subject has a history of metastatic disease, or active cancer (excluding non-melanoma skin cancer, Stage I cervical cancer, ductal carcinoma *in situ* of the breast, or Stage 0 chronic lymphocytic lymphoma) within the previous 5 years 8. Subject has any condition (*e.g.*, other skin conditions or diseases, metabolic dysfunction, physical examination findings, clinical laboratory findings) or situation (*e.g.*, vacation, scheduled surgery) that would, in the investigator's opinion, impair evaluation of a Target cNF Tumor or which exposes the subject to an unacceptable risk by study participation 9. Subject has participated in an investigational drug trial in which administration of an investigational study medication occurred within the previous 30 days. No waivers to the exclusion criteria are permitted.
Study Medication, Dosage and Mode of Administration	NFX-179 Gel or Vehicle Gel for topical application QD for 28 days to 5 Target cNF Tumors: • NFX-179 Gel 0.05% for topical administration, once daily for 28 days • NFX-179 Gel 0.15% for topical administration, once daily for 28 days • NFX-179 Gel 0.50% for topical administration, once daily for 28 days • Vehicle Gel, for topical administration, once daily for 28 days

Criteria for Evaluation	***Safety Assessments***: Adverse Events: During the study, subjects will be assessed for the occurrence of new and ongoing AEs. Descriptions of AEs will include the date(s) of onset and resolution (if resolved), maximum severity, and seriousness, action taken regarding the study drug, corrective treatment, outcome, and the investigator's assessment of causality. Safety Laboratory Tests: Routine safety laboratory tests (CBC/differential, serum chemistry, and urinalysis) will be performed at Visits 1, 2 and 5 Any out-of-range laboratory result that is considered clinically significant by the investigator will be recorded as an AE Electrocardiogram: A 12-lead ECG will be conducted at Visits 1, 2 and 5. ECG performance procedures will be standardized. The ECG results will be assessed for any clinically significant abnormality or relevant changes from baseline. Local Tolerability: Local tolerability on each Target cNF will be evaluated through assessment of selected signs (*i.e.*, erythema, edema, scabbing/crusting, vesiculation and erosion) and symptoms (*i.e.*, stinging, burning and pruritus) of local tolerability at Visits 2-5. Pregnancy Tests: All female subjects who are women of childbearing potential will have a urine pregnancy test performed at Visits 1, 2 and 5. Physical Examination and Vital Signs: A physical examination will be performed at the Screening visit. Vital signs will be measured at Visits 1, 2, 5 and 7. ***Efficacy Assessments***: Target cNF Tumor volume: At select sites the investigator will measure the volume of each Target cNF Tumor using a high frequency ultrasound at Visit 2 (Baseline) and Visit 5 (end of treatment). Target cNF Tumor dimensions: The investigator will measure the diameter of each Target cNF Tumor using a ruler at Visit 1 (Screening), Visit 2 (Baseline) and Visit 5 (end of treatment). Physician's Tumor Assessment: The investigator will assess a Physician's Tumor Assessment, for each Target cNF Tumor at Visit 2 (Baseline) and Visit 5 (end of treatment). Subject's Self-Assessment: Each subject will assess a Subject's Self-Assessment, a Patient Reported Outcome, for each Target cNF Tumor at Visit 2 (Baseline) and Visit 5 (end of treatment). ***Other Assessments***: Demographics: Demographic characteristics will be reported for each subject will be collected at Visit 1 (Screening).

	PK: At select sites blood samples for assessment of NFX-179 levels will be collected at Visit 2 (Baseline), prior to the first study medication application, and at Visit 5 (end of treatment). Tumor excisions: At Visit 5 (end of treatment) the investigator will excise all 6 Study cNF Tumors to assess for p-ERK levels. Standardized Photography: Standardized 3-dimensional color photographs to determine the volume, above the surrounding skin, of the 5 Target cNF Tumors will be taken at Visit 1, Visit 2 (Baseline) and Visit 5 (end of treatment).
Statistical Methods	All subjects who are randomized and dispensed study drug will be included in the intent-to-treat (ITT) analysis set. All subjects who are randomized, receive at least 1 confirmed dose of study drug, and have at least 1 post-baseline safety assessment will be included in the safety population. Last observation carried forward (LOCF) will be used to impute efficacy data that are missing post-baseline through Visit 5 (Week 4). Analyses will also be carried out on observed data. No imputations will be made for missing safety data. No adjustments for multiplicity will be made. ***Safety***: Safety Endpoints: • Adverse events • Local tolerability including: o erythema o edema o scabbing/crusting o vesiculation o erosion o stinging o burning o pruritis • NFX-179 concentration levels in plasma • Clinical laboratory assessments ***Statistical Analyses:*** The first primary objective is to determine the pharmacodynamic activity of NFX-179 Gel as defined by suppression of p-ERK levels in the Target cNF Tumors in the NFX-179 Gel treatment groups as compared to Vehicle Gel group after 28 days of QD treatment. This will be assessed by analyzing p-ERK levels at Visit 5 (Week 4). ANOVA models at the subject level will be used to compare mean p-ERK level between each active treatment group and the vehicle treatment group, with treatment as the independent factor. Within each subject, both the mean and the median p-ERK levels will first be calculated across all 5 tumors. The within-subject mean levels will be analyzed in the primary ANOVA model. The within-subject median levels will also be analyzed in a second ANOVA model to assess whether individual tumor outlier values may have an influence on the analysis. Mixed-model repeated measures ANOVA models will also be used to analyze p-ERK data at the tumor level. In addition, p-ERK levels from the untreated tumor for each subject will be used

	in exploratory analyses to determine whether the inclusion of this reference level in analysis models may increase sensitivity. The second primary objective is to determine the safety and tolerability of treatment with NFX-179 Gel (0.05%, 0.15% and 0.50%) or Vehicle Gel applied QD for 28 days of treatment. The frequency of dermal safety and tolerability assessments including pain/burning, pruritus, erythema, edema, scabbing/crusting, and vesiculation/erosion will be summarized by treatment group and visit. All data will be listed. The third primary objective of evaluating adverse events will be based on summaries and listings of adverse events by treatment group, as described below under Safety Analyses. The first secondary objective is to determine the clinical efficacy of NFX-179 Gel defined as the percent change in neurofibroma volume after 28 days of treatment based on tumor size derived from high frequency ultrasound measurements and 3D photographs. This will be analyzed analogously to the first primary endpoint. Analyses will be conducted both on the tumor level and the subject level, as planned for the first primary endpoint. The second secondary objective is to measure systemic exposure of NFX-179 gel during the clinical study. Plasma concentrations of NFX-179 will be summarized at Visit 5 (Weeks 4) by treatment group. The third and fourth secondary objectives are to determine the clinical efficacy of NFX-179 Gel defined as the change from baseline in the Subject's Self-Assessment grade and Physician's Tumor Assessment grade, separately, after 28 days of treatment. Appropriate mixed-model ANCOVAs will be used to compare, for each treated tumor, change from baseline grade between treatment groups, with treatment as the independent factor, baseline value as the covariate, and subject as the repeated factor. ANCOVA models will also be performed on the subject level after first calculating the within-subject mean values across treated tumors. In addition, for each assessment dichotomized analyses will be conducted on the proportion of tumors achieving a grade of Clear, and also the proportion of tumors achieving a grade of either Clear or Almost Clear, after 28 days of treatment. Treatment groups will be compared to vehicle using appropriate mixed-model logistic regression, as well as a Chi-square analysis of within-subject averaged results across tumors. ***Safety Analyses:*** Subjects will be assessed for the occurrence of new and ongoing AEs. Descriptions of AEs will include the dates of onset and resolution (if resolved), maximum severity, seriousness, and action taken regarding the study drug, corrective treatment, outcome, and investigator's assessment of causality. All AEs will be recorded and classified using terminology from the Medical Dictionary for Regulatory Activities (MedDRA). All reported treatment-emergent AEs (TEAEs), defined as any AE with an onset on or after the date of first study drug application, will be summarized by treatment group, the number of subjects reporting TEAEs, system organ class, preferred term, severity, and relationship to study drug. When summarizing TEAEs by severity or relationship to study drug, each subject will be counted only once within a system organ class or a preferred term using the event with the greatest severity or causality, respectively, within each category. All reported serious adverse events (SAEs) will be summarized by treatment group, the number of subjects

	reporting SAEs, system organ class, preferred term, severity, and relationship to study drug. All information pertaining to AEs noted during the study will be listed by subject and will include a verbatim description of the event as reported by the investigator, as well as the preferred term, system organ class, start date, stop date (if stopped), seriousness, severity, action taken regarding the study drug, corrective treatment, outcome and relationship to the study drug. In addition, a listing of subjects who prematurely discontinue from the study due to AEs will be provided as well as a listing of subjects who reported an SAE. Changes from baseline in safety laboratory values and vital sign measurements will be summarized with descriptive statistics for each treatment group at all applicable study visits. Shift tables will be presented for changes in safety laboratory values to summarize laboratory test results collected at Visits 1 (Screening), 2 (Baseline) and 5 (End of Treatment). Normal ranges established by the central laboratory will be used to determine the shifts. A listing of all out-of-range laboratory test results at any assessment time point will also be provided. Determination of clinical significance for all out-of-range laboratory values will be made by each investigator and included in the listing. In addition, a listing of all clinically significant laboratory test results will be provided.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
cNF	Cutaneous neurofibromas
CR	Clinically Relevant
CRF	Case Report Form
EC	Ethics Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICH	International Council for Harmonization
IRB	Institutional Review Board
LDH	Lactic Dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
NCR	Not Clinically Relevant
NF1	Neurofibromatosis type 1
NFlection	NFlection Therapeutics
PK	Pharmacokinetic
PRO	Patient Reported Outcome
PTA	Physician's Tumor Assessment
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	Subject Identifier
SOC	System Organ Class
SOP	Standard Operating Procedure
SSA	Subject's Self-Assessment
TEAE	Treatment Emergent Adverse Event
US	United States
WHO	World Health Organization

1 INTRODUCTION

Neurofibromatosis type I (NF1) is an autosomal dominant development syndrome caused by germline mutations in the NF1 gene on chromosome 17. The NF1 gene is responsible for production of a protein called neurofibromin that is involved in the RAS pathway (RASopathy) and which is needed for normal function in many human cell types. NF1 is one of the most common genetic disorders, is not limited by a person's race or sex, and causes tumors along the nervous system which can grow anywhere on the body.

A mutation or deletion of one allele of the NF1 gene is enough for the development of NF1, although presentation varies widely. It is an age specific disease; most signs of NF1 occur as the person ages and has hormonal changes. As of 2015, there were at least 100,000 people in the U.S. who have been diagnosed with NF1. A common symptom of NF1 are benign skin tumors called neurofibromas.

The severity of NF1 varies widely, and little is known about what causes a person to have more severe or less severe cases. Sixty percent of people with NF1 have mild cases, with few signs and symptoms that have very little effect in their day-to-day lives. Forty percent of NF1 patients have moderate to severe cases, which manifest as several signs and symptoms that affect the person's quality of life. Even in this last group, signs and symptoms are rarely life-threatening.

Cutaneous neurofibromas are a common manifestation of NF1. The gene product neurofibromin negatively regulates RAS activity and acts as a tumor suppressor by preventing the activation of downstream signaling pathways such as the RAF/MEK/ERK pathway.
NFlection.

1.1 Study Rationale

NFlection tested the efficacy of a topical formulation of a novel MEK inhibitor (NFX-179) in an *ex vivo* model by using human cutaneous neurofibroma explants. Dose dependent suppression of the shared biomarker, p-ERK, by NFX-179 (0.5%, 1.0%, 1.5%) was observed in human cutaneous neurofibroma explants. p-ERK suppression was not observed in neurofibroma explants treated with the vehicle formulation.

Western Blot analysis of p-ERK level showed complete suppression of p-ERK by all three dosages of NFX-179 in the top sections of the explant and a dose dependent suppression in the bottom sections.

Taken together, these data clearly demonstrate that NFX-179 (0.5%, 1.0%, 1.5%) can penetrate human epidermis and suppress p-ERK in human cutaneous neurofibroma explants.

This clinical study is designed to evaluate the safety and efficacy of NFX-179 after 28 days of once-daily (QD) application in subjects with NF1 tumors.

1.2 Background

1.2.1 Nonclinical Experience

NFX-179 was well tolerated in the rat following a single oral administration of doses up to 1000 mg/kg. Following multiple dosing for up to 21 days NFX-179 was well tolerated at up to 300 mg/kg/day (the highest dose tested).

In the rat, following subcutaneous administration NFX-179 was well tolerated with single doses up to 300 mg/kg. Following multiple dosing for up to 14 days, one main study animal (100 mg/kg) and one TK animal (300 mg/kg) were pre-terminally euthanized on Day 12 due to poor clinical condition of the skin. Lesions, lumps and discoloration were noted at the dosing site. Macroscopic examination revealed dermal dark discoloration with numerous red dermal scabs at last dosing site associated with thickening and SC mottling, but the cause of poor condition could not be identified.

After a single subcutaneous dose, the systemic exposure was low relative to oral administration and increased in a less than proportional manner with dose. The very low exposure suggests that the dose remains in the skin as a depot and may be released very slowly.

Over 21 days of subcutaneous administration there was an increase in exposure so that it was similar to that seen following repeated oral administration.

In a 7-day dermal study, NFX-179 (0, 0.15, 1.0 and 1.5%) were administered at a dose volume of 1 mL/kg/dose. In this study there was very slight erythema and very slight edema were noted in a single animal at both 1% and 1.5% formulations on Days 7 and 8.

Systemic exposure following dermal administration was low after single dose increasing on repeat dosing.

NFX-179 was assessed in a 28-day study. Minipigs received 0, 0.5, 1.0 or 1.5% at a dose volume of 2 mL/kg/day. Following the onset of dermal lesions and clinical signs in the majority of the study animals (including controls), treatment of the animals was stopped following 5 to 9 days of dosing (depending on replicates). The observations recorded included but were not limited to slight to severe erythema; brown skin rash and discoloration; elevated body temperature; elevated neutrophil counts. These clinical signs were seen in all animals, including controls receiving vehicle only.

The clinical observations were considered to be due to the inherent irritancy of the vehicle formulation (1+ erythema) which caused unexpected reactions and then due to residency of NFX-179 in the skin and associated pharmacological effects reducing the ability of the skin to recover from the reactions to the vehicle.

Details of the non-clinical studies for NFX-179 are presented in the Investigators Brochure.

1.2.2 Clinical Experience

No human clinical studies have been conducted with NFX-179.

1.3 Risk Benefit Assessment

Detailed information about benefits and potential risks of NFX-179 may be found in the Investigators Brochure (IB). The benefit risk ratio is considered favorable.

2 STUDY OBJECTIVES AND ENDPOINTS

The objectives of this study include:

- Primary objectives:
  - To determine the safety and tolerability of treatment with NFX-179 Gel 0.05%, 0.15% and 0.50% when applied once daily (QD) for 28 days
  - To determine the pharmacodynamic activity of NFX-179 Gel as defined by suppression of phospho-ERK (p-ERK) levels in defined cutaneous neurofibroma (cNF) tumors for each NFX-179 Gel group compared with the Vehicle Gel group after 28 days of QD treatment.
- Secondary objectives:
  - To determine the effect of NFX-179 Gel defined as the percent change in neurofibroma volume after 28 days of QD treatment based on tumor volume derived from ruler measurements, ultrasound and digital imaging
  - To determine the effect of NFX-179 Gel on the change from baseline in the Subject's Self-Assessment grade after 28 days of QD treatment
  - To determine the effect of NFX-179 Gel on the change from baseline in the Physician's Tumor Assessment grade after 28 days of QD treatment.

The endpoints being evaluated include:

- Primary endpoints include:
  - Pharmacodynamic activity of NFX-179 Gel as defined by suppression of p-ERK levels in Target cNF Tumors in each NFX-179 Gel group compared with the Vehicle Gel group after 28 days of QD application
  - Safety and tolerability of treatment with NFX-179 gel (0.05%, 0.15% and 0.50%) when applied QD for 28 days
  - Assessment of adverse events (AEs)/serious adverse events (SAEs).
- Secondary endpoints include:
  - Percent change in cNF tumor volume after 28 days of QD application
  - Systemic exposure of NFX-179 Gel during the clinical study after 28 days of QD application
  - Change in Subject's Self-Assessment grade after 28 days of QD application
  - Change in Physician's Tumor Assessment grade after 28 days of QD application.

3 STUDY OVERVIEW

3.1 Study Design

This study of NFX-179 is a double-blind, randomized, vehicle-controlled, parallel-group study, evaluating safety, tolerability, pharmacokinetics and pharmacodynamic activity in subjects with cutaneous neurofibromas.

At Visit 1, the investigator will identify 6 cNF tumors (Study cNF Tumors) that fulfill the enrollment criteria. The 5 Target cNF Tumors are for treatment with the assigned study

medication. One of the 5 Target cNF Tumors must be on the face, and 4 Target cNF Tumors must be on the anterior trunk or upper extremities. The 1 Untreated cNF Tumor must be on the anterior trunk or upper extremities and **will not receive study medication applications**. At select sites the Target cNF Tumors will be measured using high frequency ultrasound to determine volume throughout the study. The assigned study medication will be applied the Target cNF Tumors once daily (QD) during the 28-day treatment period.

At Visit 2 (Day 1) eligible subjects will be randomized and will begin the 28-day QD treatment period.

Subjects will be seen at Visits 3 and 4 (Days 8 and 15) for follow-up visits during the treatment period. At Visit 5 (Day 29) subjects will be seen for the last treatment period visit, will have the 5 Target cNF Tumors and the 1 Untreated cNF Tumor excised 4 (±1) hours after the last study medication application, and will start a 28-day no treatment follow-up period. Subjects will be seen at Visit 6 (Day 36-50) for follow-up care of the Visit 5 excision wounds. Subjects will be seen for Visit 7 (Day 57) for an end of study visit.

No topical treatments other than the study medication will be applied to any of the Study cNF Tumors for the duration of the study.

3.2 Scientific Rationale for Study Design

NFX-179 is formulated as a gel for topical administration. Since application of a gel can be expected to have an effect on the treated cNF tumors, use of a vehicle gel is deemed necessary in this study to differentiate any effects due to the vehicle from the active substance. Use of a vehicle also allows to control any effect that participation in the clinical study may introduce as a result of a more careful or otherwise different management of the cNF. Finally, use of a vehicle controls bias in those safety and efficacy assessments that rely on subjective judgement by the subject or the investigator.

Systemic absorption of NFX-179 following single topical application to the 5 Target cNF Tumors is expected to be extremely low, based on serum values observed in animal studies presented in the IB. However, to confirm that systemic exposure of NFX-179 is low in human

subjects as well, blood samples will be taken to assess NFX-179 serum levels throughout the study.

3.3 Dose Selection Rationale

The selection of dose, dosing interval and duration has taken into account the results of the nonclinical pharmacology, PK and toxicology studies, which are presented in the IB.
The QD topical application frequency at the proposed concentrations is expected to result in continuous exposure at efficacious levels in a safe range. Further details on the dose selection rationale are provided in the NFX-179 IB.

3.4 End of Study Definition

The end of the study is defined as the date of the last visit of the last subject in the study globally.

If NFlection, Medical Monitor or designee, monitor, or appropriate regulatory officials indicate that the study should be halted or that a study site should be terminated, this action may be taken after appropriate consultation between NFlection and the appropriate parties. NFlection has the right to terminate this study at any time for any reason. Conditions that may warrant termination of the study include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study.
- A decision by NFlection to suspend or discontinue testing, evaluation, or development of the product.

3.5 Study Duration

The duration of study participation for each subject is anticipated to be a maximum of 88 days. Including a 28-day screening period, a 28-day treatment period and a 28-day no-treatment follow-up period. The last study visit has a window of ±4 days.

The planned duration of the study from first subject first visit to last subject last visit is planned to be approximately 64 weeks. The study end date is the date of the last subject's last visit.

4 STUDY POPULATION

This study will enroll male and female subjects aged 18 years and older, with a clinical diagnosis of NF1.

4.1 Number of subjects

Approximately 56 subjects will be randomized to 1 of the 4 study medication treatment groups at approximately 6 United States (US) investigational centers with the goal of at least 48 subjects completing the study.

4.2 Study Entry Criteria

4.2.1 Inclusion Criteria

In order to be eligible to participate in this study, the subject must fulfill all the following criteria:

1. Subject is at least 18 years of age
2. Subject must provide written informed consent prior to any study procedures
3. Subject must have a clinical diagnosis of NF1
4. Subject has 6 Study cNF Tumors (5 Target cNF Tumors [1 on the face; 4 on the anterior trunk or upper extremities] that will be treated with the assigned study medication;1 Untreated cNF Tumor on the anterior trunk or upper extremities) that each meet the following criteria:
    - Has, in the investigator's opinion, a clinically typical appearance
    - Is dome shaped
    - Is not pedunculated
    - Is a discrete tumor
    - Is not irritated
    - Is not in an area subject to repeated trauma (*e.g.*, area that is shaved, on the beltline, under a bra strap, etc.)
    - Does not have an active cutaneous infection
    - Has a diameter that is ≥5mm and ≤10mm
    - Has a height of ≥2mm
    - Is, when centered in the center of the provided template, the only cNF tumor visible
    - Is not within 5mm of the orbital rim.
5. Subject is willing to have the 5 Target cNF Tumors and the 1 Untreated cNF Tumor excised at the end of the treatment period
6. Subject is willing to have hair in the area surrounding the Target cNF Tumors shaved, if necessary, to obtain photographs
7. Subject is willing to minimize exposure of each Target cNF to natural and artificial ultraviolet radiation

8. Subject is willing to forego treatment of the Target cNF Tumors, except protocol specified therapy, during the study
9. Female subjects who are women of childbearing potential must have a negative urine pregnancy test result and be willing to use a protocol approved, contraceptive method for the duration of the study
10. Subject is willing and able to follow all study instructions and to attend all study visits.

No waivers to the inclusion criteria are permitted.

4.2.2 Exclusion Criteria

A subject who meets any of the following criteria will be excluded from participation in this study:

1. Subject has applied any of the following topical products in the previous 30 days on or in proximity to any Study cNF Tumor that, in the investigator's opinion, impairs evaluation of any the tumor or which exposes the subject to an unacceptable risk by study participation:
    - Corticosteroids
    - Retinoids (*e.g.*, tazarotene, tretinoin, adapalene)
    - > 5% of an alpha-hydroxy acid (*e.g.*, glycolic acid, lactic acid)
    - Fluorouracil
    - Imiquimod
2. Any Study cNF Tumor has ever been treated with an MEK inhibitor or a BRAF inhibitor
3. The subject has used any of the following systemic medications in the noted time period:
    - Retinoids (*e.g.*, etretinate, isotretinoin) within the previous 90 days
    - MEK inhibitors within the previous 180 days
    - BRAF inhibitors within the previous 180 days
4. Subject has a history of hypersensitivity to any of the ingredients in the study medications
5. Subject has any known intercurrent illness or physical condition that would, in the investigator's opinion, impair evaluation of a Target cNF Tumor or which exposes the subject to an unacceptable risk by study participation
6. Subject has, in the investigator's opinion, clinically relevant history of liver disease, including viral hepatitis, current alcohol abuse, or cirrhosis
7. Subject has a history of metastatic disease, or active cancer (excluding non-melanoma skin cancer, Stage I cervical cancer, ductal carcinoma *in situ* of the breast, or Stage 0 chronic lymphocytic lymphoma) within the previous 5 years
8. Subject has any condition (*e.g.*, other skin conditions or diseases, metabolic dysfunction, physical examination findings, clinical laboratory findings) or situation (*e.g.*, vacation, scheduled surgery) that would, in the investigator's opinion, impair evaluation of a Target cNF Tumor or which exposes the subject to an unacceptable risk by study participation

9. Subject has participated in an investigational drug trial in which administration of an investigational study medication occurred within the previous 30 days.

No waivers to the exclusion criteria are permitted.

4.3 Subject identifier (SI)

At Visit 1 (Screening) the investigator or designee will assign a unique four-digit subject identifier (SI) to each subject.

The SI format will be NN-NN where the first 2 digits are the investigational center site number (using leading zeroes as appropriate) assigned by NFlection. The final 2 digits are the subject number and must be assigned in ascending numerical order, without omitting or repeating any number, starting with 01 at each investigational center. For example, the SI for the second subject that signs an informed consent at site number 04 would be 04-02.

The subject will be identified using the SI in all study documentation for the duration of the study.

4.4 Screen Failures

A subject who consents to participate in the study but is not subsequently randomized to study medication will be considered a screen failure.

A minimal set of screening failure information is required to ensure appropriate reporting of screen failure subjects and to respond to queries from regulatory authorities. For detailed screening failure requirements please refer to the case report form (CRF) completion guidelines.

Individuals whose screening data do not meet the criteria for participation in this study (*i.e.*, screen failure) may be rescreened after consultation of the Medical Monitor. If the subject will be rescreened, a new SI will be created.

4.5 Early Termination Visit

The investigator should complete the following study procedures for subjects who prematurely discontinue from the study:

- For subjects who are randomized and discontinue from the study prior to Visit 5 complete the Visit 5 procedures except Study Tumor excision
- For subjects who complete Visit 5 and discontinue from the study prior to Visit 6 complete the Visit 6 procedures
- For subjects who complete Visit 6 and discontinue from the study prior to Visit 7 complete the Visit 7 procedures.

Detailed instructions for documenting subjects who prematurely discontinue from the study will be included in the CRF guidelines.

4.6 Unscheduled Visit

The investigator may see a subject for an unscheduled study visit at any time it is, in the investigator's opinion, necessary.

At unscheduled visits, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Review the study instructions with the subject
4. Schedule the next study visit.

Complete other study procedures that are, in the investigator's opinion, appropriate for the visit. Detailed instructions for documenting unscheduled visits will be included in the CRF guidelines.

4.7 Lost to Follow-up

A subject who appears to be unavailable for scheduled visits and is unable to be contacted by the study site will be considered lost to follow-up.

The following actions must be taken if a subject repeatedly fails to return to the study site for a required study visit:

- The investigator or designee must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, she/he will be considered to have withdrawn from the study.

4.8 Subject discontinuation from the study

Subjects will be informed that they are free to withdraw from the study at any time and for any reason.

The investigator may remove a subject from the study if, in the investigator's opinion, it is not in the best interest of the subject to continue the study. The investigator must remove a subject from the study if the subject experiences:

- A Serious Adverse Event the investigator defines as related to the study medication, regardless of the intensity of the event
- A non-serious adverse event the investigator defines as related to study medication that has a severity of severe and requires treatment to resolve.

Examples of other reasons subjects may be discontinued from the study are a change in compliance with an inclusion or exclusion criterion, occurrence of AEs, occurrence of pregnancy or use of a prohibited therapy. Notification of discontinuation will immediately (within 24 hours) be made to the NFlection study monitor.

All withdrawn subjects with ongoing AEs will be followed as appropriate.

The study may be discontinued at the discretion of NFlection. Some examples of reasons for

discontinuation are the occurrence of the following:

- Increased frequency, severity or duration of known AEs
- Medical, regulatory or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of subjects.

4.9 Replacement of subjects

Subject enrollment will continue until approximately 56 subjects have been randomized.

Subjects who are randomized and do not complete the study will not be replaced.

4.10 Subject instructions

At Visit 1, an investigational center staff member will dispense a Subject Instruction sheet to each subject.

During the entire duration of the study subjects must:

- Continue to use her/his routine cleansers and cosmetics
- Not apply any non-study topical products to any Target cNF within 18 hours prior to a study visit
- Avoid exposing each Target cNF to excessive natural (*e.g.*, sunlight) or artificial (*e.g.*, tanning beds) ultraviolet radiation and use her/his routine sunscreen if excessive exposure cannot be avoided
- Bring the subject instruction sheet to every visit.

During the 4-week treatment period, subjects must:

- Apply the study medication to each Target cNF as directed, once daily, with 24 (±6) hours between applications
- Not apply the study medication to any open wounds or obviously infected skin
- For Visits 3 and 4 do not apply the study medication within 18 hours prior to the visit
- **Prior to Visit 5 perform the study medication application 24 (±6) hours prior to the visit**
- Not wash or submerge a Target cNF Tumor for at least 6 hours after a study medication application
- Not apply any topical products to a Target cNF for at least 6 hours after a study medication application
- Not allow anyone else to use your study medication
- Keep the study medication away from children
- Store the study medication at room temperature
- Bring all study medication tubes to every visit
- Bring the subject instruction sheet to every visit.

4.11 Previous and Concomitant Therapies

4.11.1 Previous therapies

At Visit 1, the investigator or designee will question the subject to ensure they have not used any excluded therapies.

4.11.2 Concomitant Therapies

Concomitant therapies are any new or existing therapy received from Visit 1 until discharge from the study.

Concomitant therapies include drug (*e.g.*, prescription, over-the-counter) and non-drug (*e.g.*, chiropractic, physical therapy, energy-based treatments) therapies. Subjects will refrain from receipt of any therapy in compliance with the inclusion/exclusion criteria. Subjects should refrain from changing the use of any concomitant therapies during the study.

All new or modified concomitant therapies used during the study must be recorded.

Any new or modified concomitant therapy must be considered to determine if it is related to an AE. An AE must be reported unless the therapy is modified for non-medical reasons *(e.g.*, health insurance purposes), it is for prophylaxis (*e.g.,* vaccinations), it is for the Visit 5 excision of, or post-excision management of the Study cNF Tumors following the investigator's standard of care.

4.11.3 Prohibited therapies

Subjects must not use any systemic therapy for cNF and must not use any topical cNF therapy on the Untreated cNF Tumor and must not use any topical cNF therapy other than the study medication, on any Target cNF Tumor.

During this study, subjects are prohibited from using therapies listed in the exclusion criteria. The investigator should notify the NFlection Medical Monitor immediately (within 24 hours) if any prohibited therapies are required to ensure subject safety.

Starting at Visit 2 subjects must avoid applying any topical product to any Target cNF Tumor for at least 6 hours after any study medication application.

4.12 Study Medications

The study medications are faint yellow, water clear gels and are indistinguishable in physical appearance. Study medications must be maintained in a secure area with limited access under appropriately controlled and monitored storage conditions at each investigational center.

4.12.1 Study medication identity

Study Medication Information				
Name	NFX-179 Gel 0.50%	NFX-179 Gel 0.15%	NFX-179 Gel 0.05%	NFX-179 Gel Vehicle
Manufacturer	MedPharm Limited Unit 1, Chancellor Court, 50 Occam Road, Surrey Research Park Guildford, Surrey GU2 7AB United Kingdom			
Active ingredient	2-((2-fluoro-4-iodophenyl) amino)-*N*-(2-hydroxyethoxy)-1-methyl-1*H*-pyrrolo[2,3-*b*] pyridine-3-carboxamide			None
Active concentration	0.50%	0.15%	0.05%	0%
Storage conditions	59°F to 77°F (15°C to 25°C)			

The investigator or designee must confirm appropriate storage temperature was maintained during shipment for all study medication received and any discrepancies must be reported to NFlection and resolved before the study medication is dispensed.

4.12.2 Study medication packaging and labeling

The study medications will be packaged in identical appearing white plastic tubes that each contain 30 grams of study medication.

One Subject Kit that contains 2 tubes of study medication will be prepared for each subject.

Subject Kits will be labeled with a two-part, three-panel label. One part of the label remains affixed to the Subject Kit and the second part, a two-panel tear-off, is separated when the Subject Kit is first dispensed to the subject.

The affixed label part and the first panel of the tear-off label part show at least the following:

- Subject Kit number (Subject Kit number is also the randomization number)
- Protocol number
- Space to enter the SI
- Space to enter the date randomized
- Storage conditions
- NFlection information.

The second panel of the tear-off part is a blinded label (*e.g.*, scratch-off panel or equivalent) which when opened identifies the study medication in the Subject Kit. The blinded label should be opened only in a medical emergency.

Each study medication tube will be labeled with a one-part label that remains attached to the tube and shows at least the following:

- Subject Kit number
- Protocol number
- Tube number
- Space to enter the SI
- Space to enter the date dispensed
- Directions for use
- Investigational drug warning

4.12.3 Method of study medication assignment

Prior to the study start NFlection, or a designated third party, will generate a list of randomization numbers that will be provided to the assigned vendor for study medication labeling. The randomization number is also the Subject Kit number.

The randomization list will be stored with access limited to the appropriate personnel for the study medication labeling. The randomization list will be made available as appropriate to unblind the database.

4.12.4 Subject randomization

At Visit 2, the investigator will determine the subject's eligibility for randomization to study medication. To be eligible for randomization the subject must:

- Continue to fulfill all the inclusion criteria and none of the exclusion criteria
- If a Woman of Childbearing Potential (WOCBP), has a negative urine pregnancy test result at Visit 1 and Visit 2
- Have clinical laboratory test results for all measured analytes at Visit 1 and all results are either within the range of normal for the laboratory or, any abnormal results, are defined as Not Clinically Relevant (NCR) by the investigator
- Have Visit 1 ECG results that are defined as Normal.

The investigator or designee will assign study medication to eligible subjects by selecting the Subject Kits in chronological sequence in ascending numerical order starting with the lowest available Subject Kit number. The investigator will not omit or reuse any Subject Kit number.

4.12.5 Dispensing and collecting study medication

The study medication must be dispensed only to study subjects, only at investigational centers specified on the Form FDA 1572 (or its equivalent) and only by authorized personnel as required by applicable regulations and guidelines.

At Visit 2, after a subject is randomized, locate the Subject Kit with the lowest available number and complete the label.

Next locate the unused study medication tube with the lowest available number and complete the label. Weigh the study medication tube. Dispense one study medication tube to the subject for use in the first study medication application.

The subject must bring all dispensed study medication tubes with them to all visits.

At Visits 3 and 4, examine the study medication tubes dispensed to the subject, collect any tube that contains no useable study medication, weigh the tube and dispense a new tube, if required, following the instructions above.

At Visit 5, collect all study medication tubes from the subject and weigh each tube.30 grams (1

tube) of study medication tube is enough to complete the study medication applications for the duration of the study.

The investigational center staff member should make every effort to obtain all dispensed and unused study medication. Two documented telephone contacts followed by a registered letter to the subject are adequate follow-up efforts. If these efforts fail, the reason for the failure must be noted on the appropriate line of the study medication inventory CRF.

All unused and un-dispensed study medication should be held for inspection by NFlection's study monitor. Upon completion of the study all study medication will be returned to NFlection or a designated third party by the monitor using a traceable method.

4.12.6 Weighing study medication

An investigational center staff member will weigh each study medication tube when dispensed using the provided scale (or equivalent). The study medication container weight must be reported in grams (g) to the nearest 0.1g.

An investigational center staff member will weigh each study medication tube when returned. Study medication tubes that are examined and not collected from the subject because they contain useable study medication do not need to be weighed.

The study medication tube weights must be noted on the appropriate CRF.

The scale used to weigh the study medication tubes must be calibrated, according to the instructions provided for the scale, and the calibration documented, each day the scale is used, prior to use. Any scale that does not pass the calibration test must not be used to weigh the study medication.

4.12.7 Route of Administration, frequency and treatment period

The study medications are applied topically to each Target cNF Tumor, once daily for 28 days.

4.12.8 Study medication application

The study medications are for external, topical use on each Target cNF Tumor only and on the appropriate study subject only.

To perform a study medication application the subject should:

- Wash her/his hands before starting the application
- Avoid applying the study medication any open wounds or obviously infected skin
- Apply an amount of the assigned study medication sufficient to cover each Target cNF with a thin layer of the medication
- Gently rub in the study medication until no visible accumulation is evident
- Wash her/his hands after completing the application

The subject must apply study medication to the entire area of each Target cNF Tumor limiting exposure to the un-affected skin surrounding the tumor.

At Visit 2, an investigational center staff member will instruct the subject on the proper application technique The staff member will observe the subject's study medication application to ensure proper application technique and record the time the subject completes the application to the last Target cNF Tumor as the Application Completion Time. The staff member will monitor the subject for adverse events for at least 20 minutes after the Application Completion Time. The subjects are to continue QD application of the assigned study medication to each Target cNF Tumor for 4 weeks with 24 (±6) hours between applications.

At Visit 5, the study medication application will be performed by the subject as part of the visit procedures at the investigational center. Record the time the subject completes the application to the last Target cNF Tumor as the Application Completion Time. The study medication tubes will be collected after the application at this visit.

For Visits 3 and 4 subjects should not apply the study medication within 18 hours prior to the study visit.

Prior to Visit 5 subjects must be instructed to perform the study medication application 24 (±6) hours prior to the visit.

After completing a study medication application, a subject should not:

- Wash or submerse any Target cNF Tumor for at least 6 hours
- Apply any topical products (*e.g.*, cosmetics, emollient/moisturizer, sunscreens) to any Target cNF Tumor for at least 6 hours.

4.12.9 Dose Modification

Subjects should not modify the study medication application procedure or frequency. All application modifications must be reported on the appropriate CRF page.

If any significant study medication intolerance or safety issue occurs, after consulting with the NFlection's Medical Monitor, the investigator or designee may direct the subject to reduce the study medication application frequency. If the subject cannot perform QD applications to all Target cNF Tumors for more than 4 consecutive days, the subject must be removed from the study.

4.12.10 Accountability

The investigator or designee will maintain an accurate record of the receipt of the study medications as shipped by NFlection or designee, including the date received and the condition of the study medications. One copy of this receipt will be returned to NFlection when the contents of the study medication shipment have been verified and one copy maintained in the study file. In addition, an accurate study medication disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensing. This inventory record will be available for inspection at any time. At the completion of the study, the original inventory record will be available for review by NFlection upon request.

4.12.11 Return and disposition of study medication

At the completion of the study, all unused study medication will be returned to NFlection or designee for disposal per NFlection's or designee's written instructions.

4.13 Blinding

4.13.1 Verification of blinding

Blinding of the study medications is important for validity of this study. This study uses a double-blind design. The study medications are indistinguishable in appearance, packaging and labeling.

4.13.2 Unblinding the study medication

Blinding is important for validity of this study; however, the blind may be broken in the event of a medical emergency in which knowledge of the study medication identity is critical to the management of the subject's course of treatment. Before breaking the blind, the investigator should determine that the information is necessary (*i.e.*, that it will alter the subject's immediate course of treatment). In many cases, particularly when the emergency is clearly not study medication-related, the problem may be effectively managed by assuming that the subject is receiving active study medication without the need for unblinding.

If deemed necessary to break the blind for a study subject, attempt to contact the NFlection Medical Monitor to obtain concurrence. If it is not possible to contact the Medical Monitor beforehand, contact her/him as soon as possible after breaking the blind for a subject.

To identify a subject's study medication, locate the second panel of the Subject Kit tear-off label attached to the subject's CRF and follow the unblinding instructions on the label. Record the date of unblinding, the reason for unblinding and the initials of the investigational center staff member who performed the unblinding in the subject's CRF.

Any subject whose blind has been broken must be discharged from the study.

4.14 Study medication adherence

Adherence with the study medication application frequency will be monitored by questioning the subject regarding adherence at each visit and by measuring the weight of the study medication tubes when dispensed to the subject and when collected from the subject.

4.15 Other study supplies

In addition to the equipment and supplies provided for procedures, such as blood sampling, ultrasound measures and photography, NFlection, or designee, will provide the following items to each investigational center prior to the initiation of subject enrollment:

- Rulers for measuring each Study cNF Tumor
- Scales for weighing the study medication tubes
- Supplies for tissue excision processing and storage.

5 STUDY VISITS AND PROCEDURES

5.1 Schedule of Events

Visit	1 Screening	2 Randomization	3, 4 Treatment	5 End of treatment	6 Excision wound care	7 End of study	Protocol section
Day	**-28 to 0**	**1**	**8, 15**	**29**	**36-50**	**57**	
Informed consent	X						11.3
Subject identifier (SI)	X						4.3
Inclusion/exclusion criteria	X	X					4.2
Demographics	X						6.3.1
Medical history	X	X					6.1.4
Physical examination	X						6.1.4
Vital signs	X	X		X		X	6.1.6
Clinical laboratory samples	X	X		X			6.1.2
Electrocardiogram (ECG)	X	X		X			6.1.8
Study/Target cNF Tumor location identification	X	X	X	X			5.3
Study/Target cNF Tumor dimensions	X	X		X			6.2.1
Local tolerability assessment (LTA)		X	X	X			6.1.3
Physician's Tumor Assessment (PTA)		X		X			6.2.4
Subject's Self-Assessment (SSA)		X		X			6.2.3
Ultrasound (select sites)		X		X			6.2.2
Photography	X	X		X			6.3.4
Study cNF Tumor excision				X			6.3.3
Excision wound care					X		6.3.3
Pharmacokinetic samples (select sites)		X		X			6.3.2
Urine pregnancy test	X	X		X			6.1.7
Screening	◆—						5.2.1
Randomization		X					4.12.4
Dispense/collect/weigh study medication		X	X	X			4.12.5/ 4.12.6
Once-daily (QD) application		◆—	—	—◆			4.12.8
No treatment follow-up					◆—	—◆	5.2.4
Subject instructions	X	X	X	X	X		4.10
Concomitant therapies	X	X	X	X	X	X	4.11.2
Adverse events		X	X	X	X	X	7

5.2 Study Visits

A written, signed informed consent form (ICF) must be obtained from each subject prior to performing any study related procedures and/or evaluations.

For Visits 3 and 4 subjects should not apply the study medication within 18 hours prior to a study visit.

Prior to Visit 5 subjects must be instructed to perform the study medication application 24 (±6) hours prior to Visit 5.

5.2.1 Visit 1/Day -28 to 0 (Screening)

At this visit, the investigator or designee will:

1. Review and explain the nature of the study to the subject, obtain the subject's signature on the appropriate approved ICF and Health Insurance Portability and Accountability Act (HIPAA) authorization and provide a signed and dated copy to the subject
2. Assign a Subject Identifier to the subject
3. Confirm the subject meets all inclusion criteria and no exclusion criteria
4. Collect demographic and medical history information
5. Report concomitant therapies information
6. Conduct a physical examination
7. Measure vital signs
8. Perform an ECG
9. Collect samples for clinical laboratory tests
10. Perform a urine pregnancy test for women of childbearing potential (WOCBP)
11. Identify 6 Study cNF Tumors (*i.e.*, 5 Target cNF Tumors and 1 Untreated cNF Tumor)
12. Measure the dimensions of each of the 6 Study cNF Tumor
13. Take standardized color photographs of each of the 6 Study cNF Tumor
14. Review the study instructions with the subject and dispense a Subject Instruction Sheet
15. Schedule Visit 2 (Day 1) within 28 days.

5.2.2 Visit 2/Day 1 (Randomization; start of QD treatment)

This visit must occur within 28 days after Visit 1.

Subsequent study visit dates must be scheduled based on the date of this visit.

This visit may not occur until the investigator reviews the subject's Visit 1 clinical laboratory test results and ECG results.

At this visit, the investigator or designee will perform the following procedures PRIOR TO RANDOMIZATION:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report any changes as medical history or report as an AE as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Confirm the subject continues to fulfill all the inclusion criteria and none of the exclusion criteria
4. Confirm the subject has followed all study instructions
5. Perform a urine pregnancy test for WOCBP
6. Confirm the location of each Target cNF Tumor
7. Measure the dimensions of each Target cNF Tumor
8. Confirm subject is eligible for randomization, discharge subjects who are not eligible from the study.

For subjects eligible for randomization, the investigator or designee will perform the following procedures: `

1. Have the subject perform a pre-application Local Tolerability Assessment (LTA) of the symptoms for each Target cNF Tumor
2. Have the subject perform a Subject's Self-Assessment (SSA) for each Target cNF Tumor
3. The investigator will perform a pre-application LTA of the signs for each Target cNF Tumor
4. Perform a Physician's Tumor Assessment (PTA) for each Target cNF Tumor
5. Take standardized color photographs of each Target cNF Tumor
6. **At select sites only: Perform an ultrasound of each Target cNF Tumor**
7. Measure vital signs
8. Perform an ECG
9. Collect samples for clinical laboratory tests
10. Randomize eligible subjects
11. Weigh and dispense study medication
12. **At select sites only: Collect a blood sample for PK analysis prior to the first study medication application**
13. An investigational center staff member must:
    - Instruct the subject on the proper study medication application technique
    - Observe the subject's first study medication application to each Target cNF Tumor and record the Application Completion Time
    - Monitor the subject for at least 20 minutes after the Application Completion Time
14. Have the subject perform a post-application LTA of the symptoms for each Target cNF Tumor 10 (±4) minutes after the Application Completion Time
15. The investigator will perform a post-application LTA of the signs for each Target cNF Tumor 20 (±4) minutes after the Application Completion Time
16. Review the study instructions with the subject
17. Schedule Visit 3.

5.2.3 Visits 3 and 4/Days 8 and 15 (QD Treatment)

These visits must occur within the following visit windows:

- Visit 3/Day 8: 7 days (±4 days) after Visit 2
- Visit 4/Day 15: 14 days (±4 days) after Visit 2.

Subjects should not apply the study medication within 18 hours prior to these study visits.

At these visits, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Confirm the subject has followed all study instructions
4. Confirm the location of each Target cNF Tumor
5. Have the subject perform an LTA of the symptoms for each Target cNF Tumor
6. The investigator will perform an LTA of the signs for each Target cNF Tumor
7. Collect and weigh study medication
8. Weigh and dispense study medication
9. Review the study instructions with the subject
10. Schedule the next study visit.

5.2.4 Visit 5/Day 29 (End of Treatment; start of no treatment follow-up)

This visit must occur 28 days (±4 days) after Visit 2.

At selects sites performing PK sample collection at this visit:

- Subjects will be confined at the investigational center for approximately 6 hours for the collection of PK blood samples and for excision of the 6 Study cNF Tumors
- This visit must be scheduled so that the previous study medication application was completed 24 (±6) hours prior to the 0-hour PK blood sample.

At sites NOT performing PK sample collection at this visit:
subjects will be confined at the investigational center for approximately 6 hours for excision of the 6 Study cNF Tumors.

At this visit, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Confirm the subject has followed all study instructions including, at select sites, appropriate timing of the previous study medication application for PK blood sampling
4. Measure vital signs
5. Perform a urine pregnancy test for WOCBP
6. Confirm the location of the 6 Study cNF Tumors (5 Target cNF Tumors and 1 Untreated cNF Tumor)
7. Measure the dimensions of each Target cNF Tumor
8. Have the subject perform an SSA for each Target cNF Tumor
9. Have the subject perform an LTA of the symptoms for each Target cNF Tumor
10. Perform a PTA for each Target cNF Tumor
11. The investigator will perform an LTA of the signs for each Target cNF Tumor
12. **At select sites: Perform an ultrasound of each Target cNF Tumor**
13. Take standardized color photographs of each Target cNF Tumor
14. Perform an ECG
15. Collect samples for clinical laboratory tests then conduct the appropriate activities as indicated below.

At select sites performing PK sample collection at this visit:

16. Collect 0-hour blood sample for PK analysis 24 (±6) hours after the previous study medication application and prior to the Visit 5 study medication application
17. Have the subject perform the last study medication application and record the Application Completion Time
18. Have the subject perform a post-application LTA of the symptoms for each Target cNF Tumor 10 (±4) minutes after the Application Completion Time
19. The investigator will perform a post-application LTA of the signs for each Target cNF Tumor 20 (±4) minutes after the Application Completion Time
20. Collect post-application blood samples for PK analysis
21. 4 (±1) hours after the Application Completion Time excise the 6 Study cNF Tumors (5 Target cNF Tumors and 1 Untreated cNF Tumor)
22. Collect and weigh study medication
23. Review the study instructions with the subject
24. Schedule Visit 6.

At sites NOT performing PK sample collection at this visit:

16. Have the subject perform the last study medication application
17. Have the subject perform a post-application LTA of the symptoms for each Target cNF Tumor 10 (±4) minutes after the Application Completion Time
18. The investigator will perform a post-application LTA of the signs for each Target cNF Tumor 20 (±4) minutes after the Application Completion Time
19. 4 (±1) hours after the Application Completion Time excise the 6 Study cNF Tumors (5 Target cNF Tumors and 1 Untreated cNF Tumor)

20. Collect and weigh study medication
21. Review the study instructions with the subject
22. Schedule Visit 6.

5.2.5 Visit 6/Day ≥36 to ≤50 (Excision wound care)

This visit must occur 7 (±4) days after Visit 5 and 7 (±4) days prior to Visit 7.

At this visit, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report any changes as medical history or report as an AE as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Confirm the subject has followed all study instructions
4. The investigator will manage the excision wounds per her/his standard of care
5. Review the study instructions with the subject
6. Schedule Visit 7.

5.2.6 Visit 7/Day 57 (End of study, early termination)

This visit must occur 56 days (±4 days) after Visit 2 and 7 (±4) days after Visit 6.

At this visit, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs as appropriate
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes as appropriate
3. Confirm the subject has followed all study instructions
4. Measure vital signs
5. Discharge the subject from the study.

5.3 Study cNF tumor identification

At Visit 1 the investigator will identify 6 Study cNF Tumors:

- 5 Target cNF Tumors with 1 tumor on the face and 4 tumors on the anterior trunk or upper extremities
- 1 Untreated cNF Tumor on the anterior trunk or upper extremities

The Untreated cNF Tumor should be located as far as possible from the nearest Target cNF Tumor. The Untreated cNF Tumor will NOT receive any study medication applications.

For this study the anterior trunk, upper extremities and face are defined as follows:

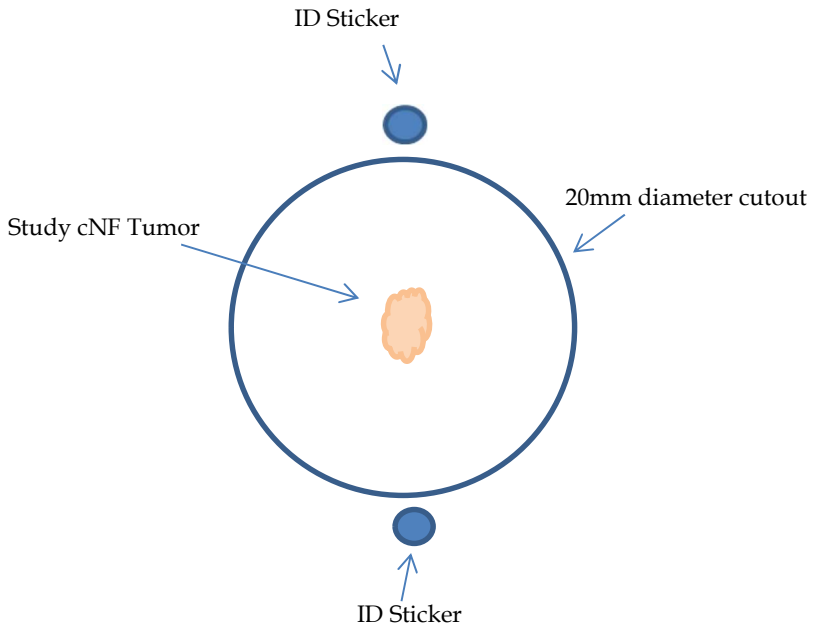
- Anterior trunk:
  - The front of the torso, excluding the neck, down to the beltline
- Upper extremities:
  - Arms from the shoulders to the tips of the fingers, including the back of the hands but excluding the palms
- Face (Target cNF Tumor #5 must be on the face):
  - Vertically from the mandibular ridge to the hairline (for subjects with a receding hair the hairline is defined by a vertical lie drawn coronally from tragus to tragus)
  - Horizontally from tragus to tragus, excluding the eyelids and areas within 5mm of the orbital rim.

At Visit 1 each of the 6 Study cNF Tumors must meet the following criteria for the subject to be enrolled and at Visit 2 the 5 Target cNF Tumors must meet to the following criteria for the subject to be randomized:

- Has, in the investigator's opinion, a clinically typical appearance
- Is dome shaped
- Is not pedunculated
- Is a discrete tumor
- Is not irritated
- Is not in an area subject to repeated trauma (*e.g.*, area that is shaved, on the beltline, under a bra strap, etc.)
- Does not have an active cutaneous infection
- Has a diameter that is ≥5mm and ≤10mm
- Has a height of ≥2mm
- Is, when centered in the center of the provided template, the only cNF tumor visible
- Is not within 5mm of the orbital rim.

At Visits 1, the investigator and an investigational center staff member will identify the 6 Study cNF Tumors using the provided template.

Number the Target cNF Tumors starting with 1 proceeding up to 5 with no number omitted or reused. Identify the Untreated cNF Tumor with the designation "UT". For identification in the study photographs place 2 appropriately colored Identification (ID) Stickers in the small template cutouts approximately 180 degrees opposite each other with the Study cNF Tumor centered in large circular template cutout (diagram not to scale):



Write the Study cNF Tumor Identifier on one of the ID stickers.

The Study cNF Tumor Identifier/ID sticker color relationships are:

- Target cNF Tumor #1/white ID stickers
- Target cNF Tumor #2/red ID stickers
- Target cNF Tumor #3/yellow ID stickers
- Target cNF Tumor #4/blue ID stickers
- Target cNF Tumor #5/green ID stickers (Target cNF Tumor #5 must be on the face)
- Untreated cNF Tumor "UT"/pink ID stickers.

Both ID stickers must be visible in the Study cNF Tumor photographs.

At Visit 1 the investigator will use body charts and the photographs to document the location of each Study cNF Tumor. The investigator will record the location of each Study cNF Tumor on an appropriate body chart using the following process:

- Make a circle identifying the location of each Study cNF Tumor
- Write the Study cNF Tumor Identifier adjacent to the circle
- Record at least the following information on each body chart:
    - Protocol number
    - Subject Identifier
    - Visit number.

At Visit 1, the investigator or designee will dispense a photocopy of the body charts completed at the visit to the subject as part of the subject instructions. The investigator or designee will place the original Visit 1 body chart in the subject's study file for use at Visit 2. The investigator or designee must also mark the approximate location of each Study cNF Tumor identified at Visit 1 in the CRFs and include the Study cNF Tumor Identifier.

At Visit 2, the investigator will use the Visit 1 body charts and photographs to identify the location of the 6 Study cNF Tumors.

Between Visit 1 and Visit 2 the status of 1 or more Target cNF Tumor may change, therefore at Visit 2, the investigator will evaluate each subject to determine which of the following categories describe each Target cNF Tumor identified at Visit 1:

- All 5 Target cNF Tumors meet the protocol requirements for the subject to be eligible for randomization
- 1 or more Target cNF Tumors do not meet the protocol requirements for the subject to be eligible for randomization, but the subject **HAS** sufficient additional cNF tumors that meet the requirements to provide 5 Target cNF Tumors for randomization (Note: 1 Target cNF Tumor must be on the face and 4 Target cNF Tumors must be on the anterior trunk or upper extremities)
- 1 or more Target cNF Tumor does not meet the protocol requirements for the subject to be eligible for randomization, and the subject **DOES NOT HAVE** sufficient additional cNF tumors that meet the requirements to provide 5 Target cNF Tumors for randomization.

At Visit 2, the investigator will discharge subjects from the study who are in category 3.

At Visit 2, for subjects who are in category 1 or 2 the investigator will follow the Visit 1

procedures to identify the 5 Target cNF Tumors to be treated and the 1 Untreated cNF Tumor. The investigator will use a new set of body charts to document the location of each Study cNF Tumor. The investigator will record the location of each Study cNF Tumor on an appropriate body chart using the following process:

- Make a circle identifying the location of each Study cNF Tumor
- Write the Study cNF Tumor Identifier adjacent to the circle
- Record at least the following information on each body chart:
    - Protocol number
    - Subject Identifier
    - Visit number.

At Visit 2, the investigator or designee will collect the body charts dispensed to the subject at Visit 1 and then dispense a copy of the body charts created at Visit 2 to the subject as part of the subject instruction review.

Clearly identify the Untreated cNF Tumor on the body chart and to the subject to ensure the subject understands the Untreated cNF Tumor is not to be treated with the study medication.

The investigator or designee will place the original Visit 2 body chart in the subject's study file for use at subsequent visits.

The investigator or designee must also mark the approximate location of each Study cNF Tumor (5 Target cNF Tumors and 1 Untreated cNF Tumor) in the CRFs and include the Study cNF Tumor Identifier.

At Visit 2-5, prior to any Target cNF Tumor assessments, **an investigational center staff member other than the evaluating investigator** will use the appropriate body charts and study photographs to identify the location each Target cNF Tumor for the study evaluations. The staff member will use the tumor template and for each Target cNF Tumor center the tumor in the large circular cutout and place the 2 appropriately colored and numbered ID stickers on the subject's skin.

At Visit 5 only, the staff member will mark the location of the Untreated cNF Tumor as described above to assist the investigator with excision.

6 STUDY ASSESSMENTS

6.1 Safety assessments

6.1.1 Adverse Event Assessments

Information regarding occurrence of AEs will be captured throughout the study. The study site visits are designed to ensure any untoward events in subjects are quickly identified by the investigator and can be treated promptly. Duration (start and stop dates), severity/grade, outcome, treatment and relationship to study medication will be recorded.

6.1.2 Clinical Laboratory Evaluations

Non-fasting samples for clinical laboratory analysis will be collected by a qualified investigational center staff member at Visits 1, 2 (prior to the randomization) and 5 (prior to the study medication application at the visit). The following tests, at a minimum, will be conducted:

Chemistry Panel	**Complete Blood Count**
Albumin	Hematocrit
Alkaline phosphatase	Hemoglobin
Alanine aminotransferase (ALT)	Platelet count
Aspartate aminotransferase (AST)	Red blood cell morphology
Blood urea nitrogen (BUN)	Red blood cell count
Bicarbonate	White blood cell count
Calcium	White blood cell differential
Chloride	% & absolute
Creatinine	Basophils
Glucose	Eosinophils
Lactate dehydrogenase (LDH)	Lymphocytes
Phosphorus	Monocytes
Potassium	Neutrophils
Sodium	
Total bilirubin	**Urinalysis with Microscopic**
Total protein	
Uric acid	

The results of the clinical laboratory tests will be reported on the laboratory's standard reports. The investigator must note NCR or clinically relevant (CR) to define the clinical relevance of any result that is outside the normal range for the laboratory. The investigator must date and sign/initial every laboratory report.

The investigator must review each subject's Visit 1 laboratory reports prior to Visit 2. The subject must not be randomized if any of the Visit 1 results are outside normal range for the laboratory AND, in the opinion of the investigator, CR. The investigator must review all laboratory reports in a timely manner.

The investigator must report all laboratory results that are BOTH outside the normal range for the laboratory AND, in the opinion of the investigator, CR as medical history if found prior to the first study medication application or as an AE if found after the first study medication application begins.

6.1.3 Local Tolerability Assessment

At Visits 2-5, the investigator and the subject will assess the local tolerability on each Target cNF using the LTA. The LTA is the investigator's and the subject's assessment of the average overall severity of the signs and symptoms, respectively, associated with irritation on each Target cNF. The investigator and subject must NOT refer to any other evaluation to assist with these assessments. This is not a comparison with the assessment at any other time point.
The investigator will assess the LTA for signs using the scales below:

Erythema		
0	None	No erythema present
1	Mild	Slight red coloration
2	Moderate	Definite redness
3	Severe	Marked erythema, bright red to dusky dark red in color

Edema		
0	None	No edema
1	Mild	Slight, but definite edema
2	Moderate	Definite edema
3	Severe	Marked edema

Scabbing/Crusting		
0	None	No scabbing/crusting
1	Mild	Slight, but definite scabbing/crusting
2	Moderate	Definite scabbing/crusting
3	Severe	Marked scabbing/crusting

Vesiculation		
0	None	No vesicles
1	Mild	Small vesicles, no pustules
2	Moderate	Vesicles with pustules, transudate may be present
3	Severe	Pustules with transudate, vesicles may be present, may extend outside treatment area

Erosion		
0	None	No erosions
1	Mild	Slight erosion on treatment area
2	Moderate	Obvious erosion(s) on the treatment area
3	Severe	Marked erosion that may extend outside treatment area

The subject will assess the LTA for symptoms using the scales below:

Stinging		
0	None	No stinging
1	Mild	Slight stinging that is not bothersome
2	Moderate	Definite stinging that is somewhat bothersome
3	Severe	Intense stinging that causes definite discomfort and may interrupt daily activities and/or sleep

Burning		
0	None	No burning
1	Mild	Slight burning that is not bothersome
2	Moderate	Definite burning that is somewhat bothersome
3	Severe	Hot burning that causes definite discomfort and may interrupt daily activities and/or sleep

Pruritus		
0	None	No pruritus
1	Mild	Slight pruritus that is noticeable but not bothersome
2	Moderate	Definite pruritus that is bothersome but does not disrupt activities and/or sleep
3	Severe	Intense pruritus that is bothersome and may interrupt daily activities and/or sleep

The investigator will assess the LTA for signs according to the following schedule:

- At Visits 2 and 5, when a study medication application is performed at the investigational center, perform the LTA for each sign prior to the start of the study medication applications and 20 (±4) minutes after the Application Completion Time.
- At Visits 3, and 4, when no study medication application is performed at the investigational center, perform the LTA for each sign anytime during the visit.

The subject will assess the LTA for symptoms according to the following schedule:

- At Visits 2 and 5, when a study medication application is performed at the investigational center, perform the LTA for each symptom just prior to the start of the study medication applications and 10 (±4) minutes after the Application Completion Time
- At Visits 3 and 4, when no study medication application is performed at the investigational center, assess the LTA for each symptom over the previous 24 hours anytime during the visit.

Both the subject and the investigational center staff member will initial and date the source document to indicate the subject performed the LTA as instructed. The subject will mark the LTA grade for each symptom on each Target cNF on the appropriate source document. The staff member assisting the subject with the LTA assessment must not influence the subject's assessment.

6.1.4 Medical history

At Visit 1, the investigator or designee, for each subject will collect medical history including all medical conditions and disease states that:

- Are ongoing
- Require concomitant therapy
- Are, in the investigator's opinion, relevant to the subject's study participation

At Visit 2, prior to randomization, the investigator will update the subject's medical history.

6.1.5 Physical Examination

At Visit 1, the investigator or designee will perform a complete physical examination for the subject that will include, at a minimum, the following body systems and organs:

- General appearance
- Skin
- Abdominal
- Extremities
- Cardiovascular
- Gastrointestinal/hepatobiliary
- Musculoskeletal
- Head, eyes, ears, nose and throat
- Lymphatic
- Neurological
- Respiratory system.

6.1.6 Vital Signs

A qualified investigational center staff member will measure the following vital signs for each subject at the Visits 1, 2 (randomization), 5 and 7.

At Visit 2, the vital signs must be measured prior to randomization:

- Body temperature
- Pulse rate
- Respiration rate
- Blood pressure (systolic and diastolic) after the subject sits quietly for at least 5 minutes
- Height (at Visit 1 only)
- Weight (at Visit 1 only).

Any measure that is, in the opinion of the investigator, abnormal AND CR must be recorded as history if found prior to the start of the first study medication application at or as an AE if found after the first study medication application begins.

A systolic blood pressure >140mm Hg or a diastolic blood pressure >100mm Hg is considered abnormal and therefore must be defined as CR or NCR in the CRF.

A weight >300 lbs. is considered abnormal and therefore must be defined as CR or NCR in the CRF.

6.1.7 Urine pregnancy tests

At Visits 1, 2 (prior to randomization) and 5, the investigator or designee will perform a urine pregnancy test for subjects who are WOCBP. The urine pregnancy test kits used must have a minimum sensitivity of 25-mIU ß-HCG/milliliter (mL) of urine.

Subjects who are WOCBP must have a negative pregnancy test result at the Visit 1 to be enrolled in the study and at Visit 2 to be randomized.

If the result of any post-treatment urine pregnancy test is positive, the subject will be withdrawn from the study and the subject's pregnancy documented and followed.

6.1.8 Electrocardiogram

A 12-lead ECG will be performed by a qualified investigational center staff member at the Visits 1, 2 (prior to randomization) and 5.

The ECGs must be obtained using a 12-lead ECG with a 10mm/mV amplitude, at 25mm/sec and 5-10 second duration. To ensure a steady heart rate the subject must rest quietly in the supine position for at least 5 minutes prior to performing the ECG.

The following parameter, at a minimum, will be determined:

- Heart rate
- RR interval
- PR interval
- QRS interval
- QT and QTc.

A normal ECG is defined as:

- QTc ≤450msec for males and ≤460msec for females (use of the ECG algorithm is acceptable for this purpose)
- Heart rate that is ≥50 and ≤100 beats/minutes
- The tracing must not show any:
    - Rhythm disturbance other than a benign sinus dysrhythmia
    - Conduction disturbance including First Degree A-V Block (PR >200msec) and pre-excitation (PR <120msec)
    - Acute or chronic signs of ischemia.

Variations such as minor ST changes (*i.e.*, <0.5mm depression) and early re-polarization are considered normal.

The ECG results must be interpreted by a qualified health professional, the evaluator, and the interpretation reported either directly on the tracing or in a separate report. The evaluator and the investigator or designee may be the same person.

The investigator or a designee must review the ECG reports in a timely manner and in all cases prior to the next study visit. If an ECG result is defined as abnormal by the evaluator the investigator or designee should define the abnormality as CR or NCR. The investigator will record this information directly on the ECG tracing/report and date and initial the tracing/report.

The subject must not be randomized if the Visit 1 ECG is, in the evaluator's opinion, abnormal, regardless of the clinical relevance.

The investigator must report ECG results that are BOTH abnormal AND CR as medical history if found prior to the first study medication application or as an AE if found after the first study medication application begins.

6.2 Efficacy Assessments

The following efficacy assessments will be conducted by the appropriate investigational center staff and according to the noted schedules.

6.2.1 Target cNF dimensions

At Visits 1, 2 and 5, the investigator will measure the diameter and the height (thickness) above surrounding skin, in millimeters (mm), of each subject's 5 Target cNF Tumors to the nearest 0.1mm using the provided ruler or equivalent. The diameter is the length of the longest dimension of the Target cNF Tumor parallel to the surrounding skin. The height is the maximum height of the tumor above the surrounding skin.

At Visit 1, to be enrolled in the study, and at Visit 2, to be randomized, each subject must have 5 Target cNF Tumors with a diameter ≥5mm and ≤10mm and a height ≥2mm.

At Visit 1 only, the Untreated cNF Tumor must be measured to confirm a diameter ≥5mm and ≤10mm and a height ≥2mm.

6.2.2 Target cNF ultrasounds

At select sites at Visits 2 (randomization) and 5, an investigational center staff member other than the investigator will perform a high frequency ultrasound (HFUS) imaging on each Target cNF. The HFUS must be taken prior to the study medication application at the visit.

The HFUS results will be used to determine the volume of the Target cNF.
HFUS imaging will be performed using an appropriate HFUS system (*e.g.*, Vevo3100, GE Logiq E, etc.).

The HFUS operator will be trained by the investigator or a subinvestigator in the proper device calibration and operation. This training will be documented in the study file. Equipment, supplies, training and detailed instructions for obtaining and managing the ultrasound images will be provided to the investigational center prior to the initiation of subject enrollment.

6.2.3 Subject's Self-Assessment (SSA)

The SSA is the subject's assessment of the average overall severity of each Target cNF at a particular time point and is not a comparison with the SSA at any other time point. The subject should NOT refer to any other evaluation to assist with these assessments.

At Visits 2 and 5, each subject will assess each Target cNF Tumor using the scale below and report the one integer that best describes the average overall severity of the Target cNF Tumor. The subject must complete the SSA prior to the study medication application and prior to the PTA assessment at the visit.

An investigational center staff member other than the investigator must educate the subject on the SSA scale before each evaluation.

To perform the SSA an investigational center staff member other than the investigator will identify the Target cNF Tumor being assessed to the subject and direct the subject to assess the tumor. The

staff member must not influence the subject's assessment. The study staff member will report the SSA grade the subject indicates for the Target cNF Tumor in the source document.

This process will be repeated until the subject has assessed all 5 Target cNF Tumors.

Both the subject and the study staff member must sign/initial the source document to indicate the subject performed the SSA as instructed.

The subject and the investigator must not discuss the subject's SSA grade and the subject must complete the assessment before the investigator performs the PTA at the visit.

Subject's Self-Assessment	
Grade	**Descriptor**
0	Clear: no visible cNF tumor
1	Almost Clear: a barely visible cNF tumor that is not noticeable (or do you think "bothersome" is better here)
2	Mild: a slightly visible cNF tumor that is somewhat noticeable
3	Moderate: an obviously visible cNF tumor that is more noticeable than I prefer
4	Severe: a prominently visible cNF tumor that is completely unacceptably noticeable

6.2.4 Physician' Tumor Assessment (PTA)

The PTA is the investigator's assessment of the average overall severity of each Target cNF Tumor at a particular time point. The investigator should NOT refer to any other assessments to assist with these assessments.

At Visits 2 and 5, the investigator will conduct the PTA for each Target cNF Tumor using the scale below and report the one integer that best describes the average overall severity of each Target cNF Tumor. The investigator must complete the PTA prior to the study medication application and after the subject completes the SSA at the visit.

Physician's Tumor Assessment	
Grade	**Descriptor**
0	Clear/None: no visible cNF tumor (barely perceptible pigmentary changes may be present)
1	Almost Clear: a visible, macular (not raised above the surrounding skin), cNF tumor
2	Mild: a cNF tumor that is raised above the surrounding skin, tumor height that is less than half the diameter, tumor is soft and easily compressible
3	Moderate: a raised dome shaped cNF tumor, tumor height is greater than half the diameter, tumor is moderately firm and somewhat compressible
4	Severe: a raised dome shaped cNF tumor, tumor height is greater than half the diameter, tumor is very firm and only minimally compressible

6.3 Other Assessments

6.3.1 Demographics

At Visit 1, the investigator or designee, will collect demographic information for the subject including date of birth, race, ethnic group and sex at birth according to local regulations. For countries where local regulatory guidelines prohibit capture of full date of birth, partial date will be recorded in line with local regulations. In addition, medical and surgical history, NF1 history, and concomitant illness(es) will be recorded.

6.3.2 Pharmacokinetic blood samples

At select sites at Visits 2 and 5, blood samples for Pharmacokinetic (PK) analysis of NFX-179 and p-ERK levels will be collected by a qualified investigational center staff member.

At select sites at Visit 2, a blood sample for PK analysis will be collected at just prior to the first study medication application.

At select sites at Visit 5, blood samples for PK analysis will be collected at:

- 0-hour; taken 24 (±6) hours after the previous study medication application and just prior to the last study medication application
- 30 minutes (±2 minutes) after the Application Completion Time
- 1 hour (±4 minutes) after the Application Completion Time
- 2 hours (±4 minutes) after the Application Completion Time
- 4 hours (±4 minutes) after the Application Completion Time.

Detailed instructions for the collection, labeling, handling, processing, storage and shipment of the PK blood samples will be provided to the select investigational center prior to the initiation of subject enrollment.

6.3.2.1 Study nourishment

At Visit 5, subjects will be confined at the investigational center:

- For PK blood sampling at select sites
- For excision samples at all sites

The investigator will provide subjects with meals, snacks and beverages as appropriate, at times that do not conflict with other study-related activities. Subjects may consume water on an *ad libitum* basis throughout these visits.

6.3.3 Study cNF Tumor excision for p-ERK levels

At Visit 5, **4 (±1) hours after the Application Completion Time for the last study medication application**, the investigator will excise all 6 Study cNF Tumors (5 Target cNF Tumors and 1 Untreated cNF Tumor) to provide tissue samples to assess for p-ERK levels.

For each of the 6 Study cNF Tumors the investigator will shave excise the entire tumor and cut the tissue into 2 halves vertically ensuring that each half contains epidermis and dermis. Half the tissue sample will be immediately frozen, and half will be preserved in formalin following the instructions below:

- Frozen sample:
    - Immediately freeze half the tissue in liquid nitrogen using forceps to immerse tissue in liquid nitrogen for ~10 seconds
    - Place the frozen tissue in a 2mL screw cap cryovial
    - Immediately freeze on dry ice
    - Store the sample at -80ºC until shipment.

- Formalin preserved sample:
    - Within 30 minutes transfer half the tissue to a container prefilled with 10% neutral-buffered formalin
    - Store at room temperature until shipment.

The tissue forceps, cryovials and prefilled containers of formalin will be supplied to each investigational center.

Each sample will be shipped to a NFlection selected laboratory for evaluation of p-ERK levels. Detailed instructions for labeling, storing and shipping the tissue samples will be provided to each investigational center prior to the initiation of subject enrollment.
The investigator will manage the excision wounds following her/his standard of care. All therapies associated with the care for the excision wounds (*e.g.*, local anesthesia, topical antibiotics, etc.) must be reported as Concomitant Therapies but their use does not need to be reported as an AE. If an unexpected event that is not normally associated with the excision procedure occurs (*e.g.*, infection, an event that is out of the ordinary in severity or duration) it must be reported as an AE, and any associated therapy must be reported as a Concomitant Therapy.

At Visit 6, the investigator will see the subject for follow-up on the excision wound care.

6.3.4 Standardized photography

At Visits 1 (Screening), 2 (Baseline/Randomization) and 5, an appropriately trained investigational center staff member other than the evaluating investigator will take standardized 3-dimensional (3D) photographs of the Study cNF Tumors. The subject's identity will not be revealed in the study photographs.

The Visit 1 and Visit 2 study images are to document the location of the Study cNF Tumors at to assist with locating the tumors at subsequent visits.

At Visits 2 and 5 the photographs must be taken prior to the study medication application at the visit. These images will be used to assess the volume of the Target cNF Tumors above the surrounding skin.

The investigator must not be allowed to use the study photographs to assist with any Target cNF Tumor evaluations.
Care must be taken to ensure similar lighting, background, subject positioning relative to the camera and camera settings are used for each photograph.

Equipment, supplies, training and detailed instructions for obtaining and managing the photographs will be provided to the investigational center prior to the initiation of subject enrollment.

6.4 Total Blood Volume

The total number of venipunctures and the total volume of blood collected during the study will be limited to that need for the safety monitoring and the pharmacokinetic assessments. Refer to the laboratory manual(s) for the collection procedures and the total volume of blood collected. The total blood volume collected for each subject for the entire study will be compliant with World Health Organization guidelines. Safety laboratory tests will be prioritized if not all laboratory samples can be collected in compliance with the blood volume guidelines.

7 ADVERSE EVENTS

All subjects who apply study medication will be assessed for safety.
The investigator is responsible for monitoring the safety of the subjects who are enrolled in this study and for alerting NFlection regarding any event that seems unusual, even if this event may be considered an unanticipated benefit to the subject. The investigator is responsible for appropriate medical care of subjects during the study.

7.1 Definitions

7.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign or symptom associated with the use of an investigational product (including an abnormal laboratory finding), whether or not related to the investigational product.

Thus, any new, CR worsening of an existing sign, symptom or disease, should be considered an AE.

Worsening of the any Target cNF assessment should be reported as an AE only if the use of the study medication is interrupted or discontinued or other therapy is required to manage the event.

Every new episode or clinically relevant worsening of a chronic condition (*e.g.*, headaches, allergies, depression, and hypertension) should be reported as a separate AE, even if the condition is reported in the subject's medical history.

The investigator should, when certain, report a diagnosis rather than the signs, symptoms or clinically relevant abnormal laboratory values associated with the AE. Otherwise, signs, symptoms or abnormal laboratory values may be used to describe the AE.

An abnormality discovered (*e.g.*, clinically relevant laboratory abnormalities), prior to the first study medication application, should be reported as medical history, not as an AE.

7.1.2 Serious Adverse Event (SAE)

A Serious Adverse Event is any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Is an important medical event

The term "life threatening" refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event that hypothetically might have caused death if it was more severe.

Inpatient hospitalization is considered to have occurred if the subject is admitted to the hospital on an in-patient basis even if released the same day. Prolongation of hospitalization is defined as an additional night stay in the hospital. Hospitalization for a diagnostic test (even if related to an AE) or elective hospitalization that was planned before study enrollment are not themselves reasons for an event to be defined as a SAE.

Important medical events are those that may not be immediately life threatening, result in death, hospitalization but are clearly of major clinical significance and may jeopardize the subject or require intervention to prevent one of the outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization.

7.1.3 Unexpected Adverse Event

An unexpected AE is any AE that the investigator defines as related to a study medication, the nature of which is not consistent with the Investigator's Brochure or package insert.

7.1.4 Adverse Events of Special Interest

The following are considered AEs of special interest, based on the route of administration, toxicology profile for NFX-179: adverse events related to kidneys, liver, coagulation, and the immune system. Reference is made to the IB for further details.

7.2 Reporting Adverse Events

The investigator is responsible for ensuring that all AEs and SAEs are recorded in the subject's medical record, AE CRF, and SAE form (if applicable), and reported to NFlection. The AE CRF can be used to report both AEs and SAEs. If necessary (*e.g.*, in case of failure of the Electronic Data Capture, system or for logistical reasons), a paper SAE form may be used instead.

7.2.1 Adverse event reporting period

The investigator must start reporting non-serious AEs from the time of the subject's first study medication application until 30 days after the last study medication application. Reporting for SAEs must start when the subject signs the ICF and continue until 30 days past the subject's last study medication application, whether or not they are related to the study.

7.3 Assessment of Adverse Events

The investigator is responsible for assessing the severity, seriousness and causality of AEs.

7.3.1 Severity of Adverse Events

The investigator is to define the severity of each AE using the following definitions as a guideline. The investigator will consider the range of the possible severity of the event and identify the severity that is the most appropriate according to her/his medical judgment:

Mild – Awareness of signs or symptom, but easily tolerated

Moderate – Discomfort, enough to cause interference with usual activity

Severe – Incapacitating with inability to perform usual activity

7.3.2 Relationship to Study Medication

The investigator will determine if there is a reasonable causal relationship between the study medication and an AE or not. The investigator will use her/his best medical judgment and consider all relevant factors (*e.g.*, temporal relationship, location of the event, the subject's relevant medical history, concomitant therapies and concurrent conditions) to determine the relationship of the AE to the study medication. The investigator will define the relationship of an AE to the study medication by selecting one of the following categories:

> **Related** – There is a reasonable causal relationship between the study medication and the AE.
>
> **Not Related** – There is not a reasonable causal relationship between the study medication and the AE.

The expression "reasonable causal relationship" is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

7.4 Procedures for reporting non-serious adverse events

At each post-enrollment visit, the investigator will question the subject to elicit AEs using a non-directive question such as "Has there been any change in your health since the previous study visit?" If appropriate, based on the subject's response to non-directed questioning to elicit AEs, the investigator will follow-up with directed questions and appropriate evaluations.

Any AE noted during the reporting period must be reported in the source documents and on the appropriate AE CRF. AEs that are defined as "Not Related" to the study medications will be followed until they are resolved or until the subject's last study visit. AEs that are defined as "Related" to the study medications will be followed until they are resolved or, if not resolved after the subject's last study visit, until in the opinion of the investigator, the AE reaches a clinically stable outcome with or without sequelae.

7.5 Procedures for reporting serious adverse events

NFlection Medical Monitor
Email: nflectionsae@nflectionrx.com
24-hour telephone: (302) 559-8684

Upon becoming aware of a SAE occurring during the AE reporting period, whether or not related to the study medications, the investigator must:

1. Take the appropriate medical action to ensure the subject's safety.
2. Immediately inform the Medical Monitor of the SAE by email, ensuring that the subject information is deidentified (only subject initials and subject identifier)
3. Print a copy of the email and place in the study file.
4. Within 24-hours complete, as fully as possible, an AE CRF and an SAE form; e-mail the forms and any other relevant documentation (*e.g.*, concomitant medication CRF, medical history CRF, laboratory test results, etc.) to the Medical Monitor.
5. Monitor and document the progress of the SAE until it resolves or, if not resolved after the subject's last study visit, until in the opinion of the investigator the SAE reaches a clinically stable outcome with or without sequelae AND the investigator and NFlection Medical Monitor agree that the SAE is satisfactorily resolved.
6. Inform the NFlection Medical Monitor of SAE updates, via telephone, followed by an SAE form update sent by e-mail.
7. Comply with the appropriate regulatory requirements and NFlection instructions regarding reporting of the SAE to the responsible Institutional Review Board (IRB) or Ethics Committee (EC).

The investigator will define the severity of every AE as Mild, Moderate, or Severe using the following intensity grades definitions:

Mild – Awareness of signs or symptom, but easily tolerated

Moderate – Discomfort, enough to cause interference with usual activity

Severe – Incapacitating with inability to perform usual activity

Note the distinction between an AE with an intensity of "severe" and a "serious" AE, a severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met one of the SAE criteria.

7.6 Pregnancy

If a female subject becomes pregnant during the treatment period or within 6 months after the last study medication application, a Pregnancy Report form should be completed and submitted to NFlection within 24 hours of learning of the pregnancy:

NFlection Medical Monitor
Email: nflectionsae@nfflectionrx.com
24-hour telephone: (302) 559-8684

Abortion, whether therapeutic or spontaneous, will also be reported on a Pregnancy Report form and sent to NFlection. If the abortion meets seriousness criteria, this information will be captured on the AE CRF and SAE form.

Any congenital anomaly/birth defect in a child born to a female subject should be recorded and reported as an SAE.

7.6.1 Woman of Childbearing Potential (WOCBP)

WOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (*e.g.*, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Postmenopausal is defined as ≥12 months with no menses without an alternative medical cause. WOCBP must have a negative UPT at Visit 1 to be enrolled in the study and at Visit 2 to be randomized to study medication.

7.6.2 Protocol approved methods of birth control

The investigator will discuss the potential risk factors associated with pregnancy and the importance of maintaining a highly effective method of birth control with all WOCBP. All WOCBP must use a protocol approved method of birth control throughout the duration of the study and for 30 days after the last study medication application.

For this study protocol approved methods of birth control include:

- Women who are abstinent from reproductive sex as a matter of lifestyle (*i.e.*, women having sex with women); or who are abstinent are acceptable methods of contraception (as these methods, if followed consistently, are at least as effective as hormonal birth control pills)
- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Transdermal
    - Intravaginal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Implantable
    - Injectable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system

WOCBP must be on at least 1 protocol approved method of birth control for the following time periods prior to Visit 1:

- Abstinence (≥30 days)
- Implants (on a stable dose for ≥30 days)
- Injectables (on a stable dose for ≥30 days)
- Patches (on a stable dose for ≥30 days)
- Combined oral contraceptives (on a stable dose for ≥30 days)
- Intrauterine devices (inserted for ≥30 days).

Prior to trial enrollment the investigator must discuss with all WOCBP the potential risk factors associated with a pregnancy during the study and the importance of avoiding pregnancy during study participation. The subject must sign an informed consent form documenting this discussion. During the trial, all WOCBP will be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period).
If a subject or investigator suspects that the subject may be pregnant prior to study medication administration, the study medication must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed, the subject must not receive study medication and must be discharged from the study.

If, following the first study medication application it is determined that the subject may have been or was pregnant at the time of study medication exposure (including 30 days after the last

study medication application) the investigator must immediately notify the NFlection Medical Monitor and record the pregnancy on a pregnancy surveillance form. While not an AE or SAE, the investigator must report every pregnancy using a pregnancy surveillance form and follow the same reporting procedures as described for reporting an SAE.

Protocol-required procedures for trial discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (*e.g.*, x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome on the pregnancy surveillance form. Infants should be followed for a minimum of six weeks.

8 STATISTICAL METHODOLOGY

8.1 Sample Size Determination

This is a Phase 2a safety study designed to evaluate the safety, proof of mechanism, preliminary efficacy and systemic exposure of NFX-179. A maximum of approximately 48 subjects with confirmed EN are planned to be randomized in the study. The sample size is based on practical considerations. The proposed number of subjects takes into account the rarity of the condition and the exploratory nature of the study and is considered adequate to fulfill the objectives of the study.

8.2 Randomization

Subjects will be randomized with a treatment allocation ratio of 1:1:1:1 (NFX-179 Gel 0.05% to NFX-179 Gel 0.15% to NFX-179 Gel 0.50% to Vehicle Gel) for cNF.

8.3 Statistical Analyses

8.3.1 General Approach

All randomized and treated subjects will be assessed in terms of safety and exploratory efficacy.

Descriptive statistics will be produced on relevant screening and baseline data and on demographic characteristics. Safety and exploratory efficacy endpoints will be summarized descriptively by treatment (when applicable) and for all subjects combined and may be analyzed using appropriate parametric or nonparametric inference tests. The primary outcome measure by suppression of p-ERK levels in defined cNF in treatment groups as compared to vehicle group after 4 weeks of treatment will be assessed. A comparison between NFX-179 treated tumors and vehicle treated tumors will also be performed. Summaries and analyses will be based primarily on non-missing data. Extent of exposure using serum levels of NFX-179 will be evaluated.

Details of the proposed statistical analysis will be documented in the Statistical Analysis Plan (SAP), which will be written following finalization of the protocol and prior to any unblinding of data.

As this is an early phase clinical study, additional exploratory analyses not necessarily identified in the SAP may also be performed to support the clinical development program. Any post-hoc, or unplanned, analyses not identified in the SAP will be clearly identified in the Clinical Study Report in accordance with ICH E3.

8.3.2 Baseline Assessment

Descriptive statistics will be performed on relevant Visit 1 and Visit 2 data (*i.e.*, data collected prior to the treatment administration) and on demographic characteristics. There will be no formal comparison of baseline data, that is, no statistical hypothesis testing.

The number and percentage of subjects enrolled in each study site will be presented by diseases classification (*e.g.*, cNF generalized severe, cNF, generalized intermediate).

8.3.3 Efficacy Analyses

The first primary objective is to determine the pharmacodynamic activity of NFX-179 Gel as defined by suppression of p-ERK levels in the Target cNF Tumors in the NFX-179 Gel treatment groups as compared to Vehicle Gel group after 28 days of QD treatment. This will be assessed

by analyzing p-ERK levels at Visit 5 (Week 4). ANOVA models at the subject level will be used to compare mean p-ERK level between each active treatment group and the vehicle treatment group, with treatment as the independent factor. Within each subject, both the mean and the median p-ERK levels will first be calculated across all 5 tumors. The within-subject mean levels will be analyzed in the primary ANOVA model. The within-subject median levels will also be analyzed in a second ANOVA model to assess whether individual tumor outlier values may have an influence on the analysis. Mixed-model repeated measures ANOVA models will also be used to analyze p-ERK data at the tumor level. In addition, p-ERK levels from the untreated tumor for each subject will be used in exploratory analyses to determine whether the inclusion of this reference level in analysis models may increase sensitivity.

The second primary objective is to determine the safety and tolerability of treatment with NFX-179 Gel (0.05%, 0.15% and 0.50%) or Vehicle Gel applied QD for 28 days of treatment. The frequency of dermal safety and tolerability assessments including pain/burning, pruritus, erythema, edema, scabbing/crusting, and vesiculation/erosion will be summarized by treatment group and visit. All data will be listed. The third primary objective of evaluating adverse events will be based on summaries and listings of adverse events by treatment group, as described below under Safety Analyses.

The first secondary objective is to determine the clinical efficacy of NFX-179 Gel defined as the percent change in neurofibroma volume after 28 days of treatment based on tumor size derived from high frequency ultrasound measurements and 3D photographs. This will be analyzed analogously to the first primary endpoint. Analyses will be conducted both on the tumor level and the subject level, as planned for the first primary endpoint.

The second secondary objective is to measure systemic exposure of NFX-179 gel during the clinical study. Plasma concentrations of NFX-179 will be summarized at Visit 5 (Weeks 4) by treatment group.

The third and fourth secondary objectives are to determine the clinical efficacy of NFX-179 Gel defined as the change from baseline in the Subject's Self-Assessment grade and Physician's

Tumor Assessment grade, separately, after 28 days of treatment. Appropriate mixed-model ANCOVAs will be used to compare, for each treated tumor, change from baseline grade between treatment groups, with treatment as the independent factor, baseline value as the covariate, and subject as the repeated factor. ANCOVA models will also be performed on the subject level after first calculating the within-subject mean values across treated tumors. In addition, for each assessment dichotomized analyses will be conducted on the proportion of tumors achieving a grade of Clear, and also the proportion of tumors achieving a grade of either Clear or Almost Clear, after 28 days of treatment. Treatment groups will be compared to vehicle using appropriate mixed-model logistic regression, as well as a Chi-square analysis of within-subject averaged results across tumors.

8.3.4 Safety Analyses

Safety and tolerability will be assessed versus baseline conditions and differences between treated tumors. Descriptive statistics will be produced, where applicable. Safety assessments will be summarized across all subjects. Summaries will be provided by treatment for tumor specific safety assessments.

Extent of exposure using serum levels of NFX-179 will be evaluated.
Adverse events noted during the study will be coded to system organ classes (SOCs) and preferred terms (PTs) using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The number and percentage of subjects with pre-treatment and treatment emergent AEs (TEAEs) will be summarized by SOC and PT. TEAEs will also be presented as on-treatment and post-treatment. All AE details will be listed. As the risk profile of NFX-179 in humans is unknown and this is a first in human study, all AEs will be considered in determining the safety profile of NFX-179 unless obviously unrelated.

Descriptive summaries of laboratory assessments will be presented for each time point, for actual values and changes from baseline.

Other safety assessments, such as physical examination will also be summarized.
Prior and concomitant medications will be coded using the World Health Organization (WHO) drug dictionary. Prior and concomitant medications will be listed and summarized by

preferred drug name.

8.4 Missing Data

Summaries and analyses will be based primarily on non-missing data, with the number of non-missing observations included in the summary. Any additional methods to handle missing data will be provided in the SAP.

8.5 Sub-Group Analyses

Exploratory investigations may be carried out based on country, age, cNF classification and other factors identified in the SAP.

9. TRAINING, DATA HANDLING AND RECORD KEEPING

9.1. Training

For each investigational center, participants will be trained to the protocol, study specific procedures, and the CRFs. Those unable to attend the training must receive on-site training from an appropriately trained individual prior to participating in any of the procedures and evaluations in this study.

Clinical Research Associates (CRAs) and other applicable personnel will be trained prior to study initiation to familiarize CRAs with the disease, the Standard Operating Procedures (SOPs), the protocol and other study specific items. Team organization, communication and operational issues will also be discussed.

NFlection will provide an investigational center file to each center.

9.2. Data Collection

The investigator must maintain required records for all study subjects. Data for this study will be recorded in the subject's source document and on the eCRFs. All data on these CRFs should be recorded completely and promptly. A copy of the completed eCRFs for each subject will be retained by the investigational center.

Records of the subject's participation in this study will be held confidential except as disclosure is required by law. The study doctor, NFlection, persons working on behalf of NFlection, and under certain circumstances, the United States Food and Drug Administration and the Institutional Review Board will be able to inspect and copy confidential study-related records that identify subjects by name. Therefore, absolute subject confidentiality cannot be guaranteed. If the results of this study are published or presented at meetings, the subject's identity will not be revealed.

9.3. Data Management

Data Management activities of this study will be subcontracted. Edit checks and review processes will be performed by the sub-contractor until all data clarifications are resolved. The data will be exported to be stored in SAS datasets (or equivalent) by the sub-contractor. After all

data clarifications are resolved and subject's evaluability is determined, the database will be locked.

9.4. Study Monitoring

Before an investigational center can enter a patient into the study, a representative of NFlection will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities regarding protocol adherence, and the responsibilities of NFlection or its representatives. This will be documented in a Clinical Study Agreement between NFlection and the investigator.

During the study, a monitor from NFlection or representative will have regular contacts with the investigational center, for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational center staff is adhering to the protocol, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (*e.g.*, clinic charts).
- Record and report any protocol deviations not previously sent to NFlection
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to NFlection and those SAEs that met criteria for reporting have been forwarded to the IRB

The monitor will be available between visits if the investigator(s) or other investigational center staff needs information or advice.

9.5. Source Documentation

Investigators must keep accurate separate records (other than the CRFs) of all subjects' visits that include all pertinent study related information. A statement should be made indicating that the subjects have been enrolled in this clinical study and have provided written informed consent. Any AEs must be completely documented. Source documentation includes results of any diagnostic tests conducted during the study.

9.6. Inspection of Records

NFlection individual or designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, study medication stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

9.7. Retention of Records

The investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the test article for investigation. If it becomes necessary for NFlection or the Regulatory Authority to review any documentation relating to the study, the investigator must permit access to such records.

10. QUALITY CONTROL AND QUALITY ASSURANCE

The study is conducted under the sponsorship of NFlection in compliance with the applicable regulatory requirements as well as applicable ICH guidelines, Declaration of Helsinki, and in respect of the sponsor and/or sub-contractor SOPs for study conduct and monitoring.

Audits may be carried out by NFlection or designee, and inspections may be performed by regulatory authorities or IRB/ECs before, during or after the study. The investigator will provide the auditing/inspecting group direct access to all study records (*e.g.*, eCRFs, subject medical records, study medication dispensing records) and the investigational center study facilities. The investigator and investigational center staff will be available and will assist the auditing/inspecting groups as appropriate.

11. ETHICS

11.1. Ethics Review

This protocol, informed consent form, any information provided to subjects, subject-recruiting advertisements, and any amendments to these items will receive IRB/EC approval prior to use. The IRB/EC must receive a copy of the Investigator's Brochure, all protocol amendments, safety reports and other study related information as required by regulation or the IRB/EC procedures.

11.2. Ethical Conduct of the Study

The rights, safety and well-being of the subjects are the most important considerations in this study and take priority over the interests of society and science.

This study will be conducted in accordance with the ethical principles originating from the Declaration of Helsinki, the current ICH E6 GCP guideline, local regulatory requirements and, at US investigational centers, in compliance with the HIPAA. The study will be conducted in compliance with the IRB/EC approved version of the protocol and any applicable amendments.

11.3. Written Informed Consent

The investigator(s) at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any study procedures. The investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the subject.

11.4. Study Conduct and Protocol Amendments

With the exception of eliminating an immediate hazard to a subject, the investigator should not deviate from the protocol or implement any changes without prior written approval from the

sponsor representative or designee and prior review and documented approval from the IRB/EC.

Changes that involve only logistical or administrative changes are allowed. The investigator should document and explain any deviation from the protocol. A protocol deviation is a non-adherence to protocol-specific study procedures or schedules that does not increase the risk to a study subject and does not affect the scientific integrity of the study.

A protocol violation is defined as any divergence from the protocol-specific study procedures or schedules that may results in an increased risk to a study subject or that affect the scientific integrity of the study. All protocol violations must be reviewed by the sponsor Medical Monitor and reported to the IRB by the investigator, as directed by the IRB-specific procedures.

11.5. Regulatory Documents

The investigator must maintain a study file containing current and complete regulatory documentation in compliance with the current ICH E6 GCP guideline. This file will be reviewed as part of the routine monitoring for this study.

11.6. Contractual Requirements

A contractual agreement will be signed between NFlection and each investigator. This document will contain supplemental information, including financial terms, confidentiality, study schedule, third party responsibility, and publication rights.