

**CLINICAL STUDY PROTOCOL AMENDMENT****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**

<b>Protocol No.</b>	NFX-179-NF1-201
<b>Protocol/Amendment Date:</b>	25-MAR-2021
<b>Amendment No.:</b>	2
<b>Supersedes:</b>	N/A
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**PROTOCOL AMENDMENT INVESTIGATOR SIGNATURE PAGE**

**Protocol Number: NFX-179-NF1-201**

**Amendment Number: 2**

**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.

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Printed Name of Investigator

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Signature of Investigator

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Date

**PROTOCOL AMENDMENT APPROVAL PAGE**

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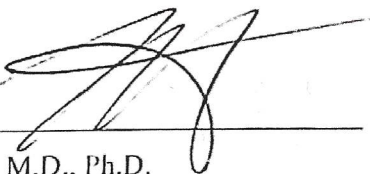
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Sponsor:	NFlection Therapeutics 714 Woodcrest Road, Wayne, PA 19087 United States



Christopher Powala  
Chief Executive Officer  
NFlection Therapeutics

03-25-2021

Date



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

3/26/21

Date

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## 1 AMENDMENT HISTORY

Revised protocol 1 footer date: 24-JUL-2020

Previous amendments: None

## 2 AMENDMENT SUMMARY

The following sections of the NFX-179-NF1-201 revise protocol with footer date 24-JUL-2020 and with a final NFlection Therapeutics approval signature date of 27-JUL-2020 are amended:

- SYNOPSIS; Statistical Methods; Statistical Analyses
- Section 8.3.1 General approach
- Section 8.3.3 Efficacy analyses
- Section 8.4 Missing data
- Section 8.5 Sub-group analyses.

## 3 AMENDMENT RATIONALE

These protocol changes are made to clarify the statistical methods and analyses that will be conducted.

## 4 PROTOCOL CHANGES

### **SYNOPSIS; Statistical Methods, Statistical Analyses:**

#### New paragraphs 1 and 2 added:

**Overview:** Analyses of efficacy parameters collected at the tumor level will primarily be conducted at the tumor level, under the assumption that tumor responses within the same subject will be predominantly statistically independent. Those parameters include p-ERK values, results based on tumor dimensions, SSA and PTA evaluations, and responder analyses based on any of these measures. This approach is consistent with the topical application of the study medication to individual tumors and the assumption of minimal systemic absorption or systemic action of the study medication. The statistical assumption of within-subject tumor response independence will be evaluated using the appropriate Shrout-Fleiss intraclass correlation based on tumor changes from baseline. For this study, that statistic is assumed to be less than 0.25. Analyses conducted at the subject level will be performed as supplementary analyses. Tumor dimension analyses will include tumor volume derived from measurements by ruler which will be the primary assessment, as well as measurements derived from ultrasound methods, and measurements based on standardized 3-dimensional (3D) photographs. Tumor volume is the

primary measurement of interest; however, the ruler measurements of tumor length and height will also be analyzed as described below.

**Contrasts:** It is anticipated that the lowest-concentration active treatment (0.05%) may have the same efficacy as vehicle. In order to fully understand the dose response results and achieve greater sensitivity to detect effectiveness for the higher-concentration treatments, all comparisons among the four treatment groups will include the following: pairwise contrasts between each active treatment group vs vehicle; a contrast between the 0.50% treatment group and the 0.05% treatment group; a contrast between the average of the 0.50% and 0.15% treatment groups vs vehicle; a contrast between the average of the 0.50% and 0.15% treatment groups vs the average of the 0.05% treatment group and vehicle; and the average of the three active treatment groups vs vehicle. In addition, all analyses and contrasts will be conducted separately for each lesion body location: Face, anterior trunk and upper extremities.

#### **SYNOPSIS; Statistical Methods:**

##### Previous paragraph 1:

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.

Changed paragraph 1, now paragraph 3:

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). An ANOVA model at the tumor 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. ANOVA models at the subject level will also be conducted as supplementary analyses. For the subject-level analyses, 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 supplementary ANOVA model. The within-subject median levels may also be analyzed in an additional supplementary ANOVA model to assess whether individual tumor outlier values may have an influence on the analysis. Supplementary mixed-model repeated measures ANOVAs may also be used to analyze p-ERK data at the subject level. In addition, p-ERK levels from the untreated tumor for each subject may be used in exploratory analyses to determine whether the inclusion of this reference level as a covariate in analysis models increases model sensitivity.

**SYNOPSIS; Statistical Methods:**Previous paragraph 3:

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.

Changed paragraph 3, now paragraph 5:

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 volume derived from ruler measurements (primary method), high frequency ultrasound measurements, and 3D photographs. These 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. In addition, for each assessment, dichotomized analyses will be conducted on the proportion of tumors achieving a reduction from baseline volume of at least 25%, and also the proportion of tumors achieving a reduction from baseline volume of at least 50%. A Chi-square analysis at the tumor level will be performed as the primary analysis between vehicle and each active treatment group, as well as a supplementary analysis at the subject level. Treatment groups may also be compared to vehicle using appropriate mixed-model logistic regression incorporation both tumor- and subject-level information, as well as a Chi-square analysis of within-subject averaged results across tumors. The mean % of responding tumors will also be analyzed with an ANOVA model.

**SYNOPSIS; Statistical Methods:**Previous paragraph 5:

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.

Changed paragraph 5, now paragraph 7:

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. These will be analyzed



analogously to the first primary endpoint. As supplementary analyses, appropriate mixed-model ANCOVAs may 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 may 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 separate Chi-square analyses. An appropriate mixed-model logistic regression may also be used, as well as an ANOVA analysis of within-subject averaged results across tumors.

### Section 8.3.1 General approach

#### Previous paragraph 1:

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

#### Changed paragraph 1:

All randomized and treated subjects will be assessed in terms of safety and exploratory efficacy. After all randomized subjects have either completed the study (Visit 7), terminated prematurely or completed treatment (Visit 5), database records will be source-document verified and locked. After that time point, there will be no further treatment with study medication. The study will then be unblinded to allow key efficacy and safety analyses to be performed. For subjects who have not completed the study, remaining visits will be conducted and data from those visits will be captured as required in the protocol, leading to a final study database lock and full study analysis.

#### Previous paragraph 3 is deleted:

~~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.~~

Previous paragraph 4:

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.

Changed paragraph 4 is now paragraph 3:

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

**Section 8.3.3 Efficacy analyses:**Previous section:

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.

Changed section:

**Overview:** Analyses of efficacy parameters collected at the tumor level will primarily be conducted at the tumor level, under the assumption that tumor responses within the same subject will be predominantly statistically independent. Those parameters include p-ERK values, results based on tumor dimensions, SSA and PTA evaluations, and responder analyses based on any of these measures. This approach is consistent with the topical application of the study medication to individual tumors and the assumption of minimal systemic absorption or systemic action of the study medication. The statistical assumption of within-subject tumor response independence will be evaluated using the appropriate Shrout-Fleiss intraclass correlation based on tumor volume changes from baseline. For this study, that statistic is assumed to be less than 0.25. Analyses conducted at the subject level will be performed as supplementary analyses. Tumor dimension analyses will include tumor volume derived from measurements by ruler which will be the primary assessment, as well as measurements derived from ultrasound methods, and measurements based on standardized 3-dimensional (3D) photographs. Tumor volume is the primary measurement of interest; however, the ruler measurements of tumor length and height will also be analyzed as described below.

**Contrasts:** It is anticipated that the lowest-concentration active treatment (0.05%) may have the same efficacy as vehicle. In order to fully understand the dose response results and achieve greater sensitivity to detect effectiveness for the higher-concentration treatments, all comparisons among the four treatment groups will include the following: pairwise contrasts between each active treatment group vs vehicle; a contrast between the 0.50% treatment group and the 0.05% treatment group; a contrast between the average of the 0.50% and 0.15% treatment groups vs vehicle; a contrast between the average of the 0.50% and 0.15% treatment groups vs the average of the 0.05% treatment group and vehicle; and the average of the three active treatment groups vs vehicle. In addition, all analyses and contrasts will be conducted separately for each lesion body location: face, anterior trunk and upper extremities.

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). An ANOVA model at the tumor 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. ANOVA models at the subject level will also be conducted as supplementary analyses. For the subject-level analyses, 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 supplementary ANOVA model. The within-subject median levels may also be analyzed in an additional second supplementary ANOVA model to assess whether individual tumor outlier values may have an influence on the analysis. Supplementary mixed-model repeated measures ANOVAs may also be used to analyze p-ERK data at the subject level. In addition, p-ERK levels from the untreated tumor for each subject may be used in exploratory analyses to determine whether the inclusion of this reference level as a covariate in analysis models may increase model 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 efficacy 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 volume derived from ruler measurements (primary method), high frequency ultrasound measurements, and 3D photographs. These 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. In addition, for each assessment, dichotomized analyses will be conducted on the proportion of tumors achieving a reduction from baseline volume of at least 25%, the proportion of tumors achieving a reduction from baseline volume of at least 30%, and also the proportion of tumors achieving a reduction from baseline volume of at least 50%. A Chi-square analysis at the tumor level will be performed as the primary analysis between Vehicle

and each active treatment group, as well as a supplementary analysis at the subject level. Treatment groups may also be compared to vehicle using appropriate mixed-model logistic regression incorporating both tumor- and subject-level information, as well as a Chi-square analysis of within-subject averaged results across tumors. The mean % of responding tumors will also be analyzed with an ANOVA model.

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. These will be analyzed analogously to the first primary endpoint. As supplementary analyses, appropriate mixed-model ANCOVAs may 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 may 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 separate Chi-square analyses. An appropriate mixed-model logistic regression may also be used, as well as an ANOVA analysis of within-subject averaged results across tumors.

#### **Section 8.4 Missing data:**

##### Previous section:

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.

Changed section:

Summaries and analyses will be based primarily on non-missing data, with the number of non-missing observations included in the summary.

**Section 8.5 Sub-group analyses:**

Previous section:

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

Changed section:

Exploratory investigations may be carried out based on country, age, cNF classification and other relevant factors.