STUDY PROTOCOL

Protocol Title: A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma

Protocol Number: ATI001-101

Phase: I/II

Date of Protocol:
Amendment 7: 29 April 2013
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Amendment 4: 13 July 2012
Amendment 3: 20 April 2012
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Original: 21 April 2011

Sponsor: ZIOPHARM Oncology, Inc.
[redacted]

Medical Monitor: [redacted]

Safety Reporting: [redacted]

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2. ABBREVIATIONS AND DEFINITIONS OF TERMS

Ad	Adenovirus	IND	Investigational New Drug
AE	Adverse Event	INXN-1001	Small molecule Activator Ligand
AL	Activator Ligand (INXN-1001)	INXN-2001	Ad-RTS-hIL-12
ALT	Alanine Transaminase	IRB	Institutional Review Board
AST	Aspartate Transaminase	kg	Kilogram
BMI	Body Mass Index	LDH	Lactate Dehydrogenase
CBC	Complete Blood Count	MCV	Mean corpuscular volume
cGCP	Current Good Clinical Practice	MDSC	Myeloid Derived Suppressor Cell
cm	Centimeter	mg	Milligram
CRF	Case Report Form	µM	Micromolar
CT	Computed Tomography	mm^3	Cubic Millimeter
CTCAE	Common Terminology Criteria for Adverse Events	MRI	Magnetic Resonance Imaging
CTLs	Cytotoxic T Lymphocytes	MTD	Maximum Tolerated Dose
CYP	Cytochrome P450	NK	Natural Killer
DCs	Dendritic Cells	PBMC	Peripheral Blood Mononuclear Cells
DLNs	Draining Lymph Nodes	PBx	Punch Biopsy
DLT	Dose-Limiting Toxicity	PK	Pharmacokinetic
ECOG	Eastern Cooperative Oncology Group	PTT	Partial Thromboplastin Time
EcR	Ecdysone Receptor	RECIST	Response Evaluation Criteria in Solid Tumors
FDA	Food & Drug Administration	RTS	[redacted]
GGT	Gamma-Glutamyl Transpeptidase	RXR	Retinoid X Receptor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor	SAE	Serious Adverse Event
HIPAA	Health Insurance Portability and Accountability Act of 1996	SOP	Standard Operating Procedure
hIL-12	Human Interleukin 12	SRC	Safety Review Committee
IEC	Independent Ethics Committee	TNF	Tumor Necrosis Factor
IFN	Interferon	Tregs	Regulatory T Cell
IL-2	Interleukin 2	ULN	Upper Limit of Normal
IL-12	Interleukin 12	vp	Viral particles

3. PROTOCOL SYNOPSIS

Title	A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number	ATI001-101
Study Sponsor	ZIOPHARM Oncology, Inc.
Objectives	The primary objective is to: • Evaluate the safety and tolerability of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) in combination with INXN-1001 (activator ligand) in subjects with unresectable Stage III or IV melanoma. The secondary objectives are to: • Inform the selection of an INXN-1001 dose(s) and regimen for further study in combination with INXN-2001. • Assess preliminary anti-tumor activity according to RECIST 1.1 criteria. • Assess anti-tumor response based on total measurable tumor burden. • Evaluate the immunological effect of study treatment in terms of cellular and humoral immune responses, as well as other biological activities in the injected tumor(s), tumor-involved draining lymph nodes (if accessible) and in the peripheral circulation. • Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells, and to assess adenovirus vector shedding in body fluids. • Assess the pharmacokinetics (PK) of INXN-1001 in subjects with unresectable stage III or IV melanoma.
Clinical Phase	I/II
Study Drugs	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (Oral Activator Ligand) Concurrent INXN-2001 and INXN-1001 dosing will be referred to as "study treatment" throughout this protocol.
No. of Subjects	Approximately 30 subjects (3 subjects per dose level cohort in Phase I and approximately 15 subjects enrolled in Phase II at a single dose level at or below the MTD), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any.
Number of Centers	Multi-center (approximately 8 to 12 centers)

Study Design

Single-arm, open label, Phase I/II dose escalation study of intratumoral injections INXN-2001 and oral INXN-1001 in subjects with unresectable Stage III or IV melanoma.

Table 1: Study Design

Study Part	Cohorts	Dose Regimen
Phase I	Dose Escalation	INXN-2001: [redacted] INXN-1001: 5, 20, 100, 160 mg
Phase II	Group 1	INXN-2001: [redacted] INXN-1001: 160 mg daily for 7 consecutive days; 21-day cycle
	Group 2	INXN-2001: [redacted] INXN-1001: 160 mg every other day for 14 days; 28-day cycle

In Phase I, four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. In each cycle, the subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001. No intrasubject dose escalation will be allowed.

[Please refer to Appendix 5 for additional study design details for the Phase I and Phase II, Group 1 portions of this study.]

In Phase II, approximately 15 additional subjects will be enrolled at a single dose level at or below the MTD. Phase II of the study will include two groups of subjects. Subjects enrolled in the Phase II portion of the study under Amendment 6 or prior will be enrolled into Group 1; subjects enrolled under Amendment 7 or later will be enrolled into Group 2.

Subjects in Group 1 will be treated with one intratumoral injection of INXN-2001 in combination with oral doses of INXN-1001 given at 160 mg daily for 7 consecutive days at the beginning of a 21-day cycle. Subjects in Group 2 will be treated with one intratumoral injection of INXN-2001 in combination with oral doses of INXN-1001 given at 160 mg every other day for 14 days at the beginning of a 28-day cycle. Subjects may receive treatment for 6 cycles.

Safety and tolerability will be assessed by the incidence and severity of adverse events as determined by NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03. A Safety Review Committee (SRC) comprised of the Medical Monitor, Principal Investigators, and sponsor representatives, will be convened to review safety information and to decide upon dose escalation and further subject enrollment.

The anti-tumor activity of study treatment will be assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 guidelines.[1] Additional assessment of anti-tumor activity will be explored based on total measurable tumor burden.[39] (Appendix 4)

Immunological and biological markers of response will include

	examinations of tumor biopsy samples, cytokine levels, peripheral blood mononuclear cells (PBMC) and antibody response to INXN-2001.
Study Population	The target population for this study is adult subjects with unresectable Stage III or IV melanoma for which is no alternative curative therapy exists. **Inclusion Criteria:** 1. Males or females of all races ≥ 18 years of age, who have provided written informed consent prior to completing any study specific procedure. 2. Unresectable Stage III or Stage IV melanoma arising from any site other than ocular melanoma. 3. A minimum of 2 accessible lesions (shortest diameter ≥ 1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥ 1.5 cm). 4. ECOG performance status of 0 or 1 (Appendix 1). 5. Adequate bone marrow, liver, and renal function, as assessed by the following laboratory requirements: a. Hemoglobin ≥ 9 g/L b. Lymphocytes > 700/ mm^3 c. Neutrophils ≥ 1,500/ mm^3 d. Platelets ≥ 100,000/ mm^3 e. Serum creatinine ≤ 1.5 x ULN f. AST and ALT ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5×ULN g. Total bilirubin < 1.5 x ULN h. International Normalized Ratio (INR) and activated partial thromboplastin time [PTT] < 1.5 x ULN, if not therapeutically anticoagulated. Subjects who are being therapeutically anticoagulated with an agent such as Coumadin (warfarin sodium) or subcutaneous heparin may be included provided there is no prior evidence of underlying abnormality in coagulation parameters, screening test results are in appropriate therapeutic range, and anticoagulation regimen is stable and closely monitored. 6. An expected survival of at least 6 months. 7. Male and female subjects must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the screening visit through 28 days after the last dose of study drug. **Exclusion Criteria:** 1. Any prior anti-cancer therapy or investigational agent within 28 days

	prior to the first dose of study drug. **NOTE:** For Phase II ONLY, if subjects received ipilimumab, a 45-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will be made. 2. Clinically significant infection requiring systemic antibacterial, antifungal, or antiviral therapy within 2 weeks of the first dose of study drug. 3. History of HIV infection. 4. Active autoimmune disease requiring steroids (> 10 mg prednisone or comparable) or other immunosuppressive therapy (eg, methotrexate, etc). 5. Documented symptomatic brain metastases. Screening for brain lesions by CT or MRI is not required for all potential subjects; however, if there are any neurological signs or symptoms consistent with brain metastases, then a brain CT or MRI should be performed as clinically indicated. 6. Any medications that induce, inhibit or are substrates of CYP450 3A4 within 7 days prior to the first dose of study drug. 7. Prior history of hematopoietic stem cell transplant or organ allograft. 8. Other concurrent clinically active malignant disease, with the exception of other cancers of the skin. 9. Females who are nursing or pregnant. 10. Subjects who have a history of hypersensitivity that may relate to any component of the study drugs, eg, to benzoic acid [redacted]. 11. Unstable or clinically significant concurrent medical condition that would, in the opinion of the investigator, jeopardize the safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, unstable angina, congestive heart failure, recent (within 2 months of screening) myocardial infarction, ongoing maintenance therapy for life-threatening ventricular arrhythmia, uncontrolled asthma, evidence of hepatic pathology due to or consistent with infection with a chronic hepatitis virus, uncontrolled major seizure disorder, or electrolyte imbalances. 12. Local infection at site of injectable lesion requiring anti-infective therapy within 2 weeks of the first dose of study drug.
Dose and Schedule	*[Please refer to Appendix 5 for the dose and schedule of subjects enrolled under the Phase I and Phase II, Group 1 portions of this study.]* Phase II, Group 2: All subjects will receive intratumoral injections of INXN-2001 [redacted]

	on Day 1 of a 4-week cycle. The subjects will also receive a single oral dose of INXN-1001 every other day for 14 days starting on Day 1 of each cycle. Subjects may receive up to 6 cycles of study treatment. INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. **Note:** One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001. If only 2 accessible lesions are present at screening and the single injected lesion can no longer support the injection volume at subsequent cycles, then the medical monitor should be contacted for a discussion.
Route of Administration	INXN-1001: Taken orally in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal. INXN-2001: Intratumoral injection on the first day of each cycle into one accessible tumor lesion or tumor-involved (palpable) draining lymph node when necessary. Radiologically-guided injections are permitted under BL2 conditions. The injection should be administered 3 hours ± 30 minutes after the first dose of INXN-1001.
Duration of Subject Participation	*[Please refer to Appendix 5 for the study duration for subjects enrolled under the Phase I and Phase II, Group 1 portions of this study.]* Phase II, Group 2 subjects' participation in this study will last approximately 8 months, including: • 30 day screening period. • 6 cycles (24 weeks) of study treatment. • Post-Treatment Safety Assessment visit performed 28 days after the last dose of INXN-1001. • Follow-Up Tumor Assessment visit performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. In addition, subjects who discontinue or complete study treatment in the absence of rapid clinical deterioration and without objective evidence of confirmed immune-related progressive disease (irPD) should continue to be followed until irPD has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.
Safety Evaluations	Safety parameters will include serious adverse events (SAEs), adverse events (AEs), physical examinations, electrocardiograms (ECGs), vital signs, clinical laboratory evaluations, medical history, and prior/concomitant medications.
Efficacy Evaluations	Efficacy will be evaluated as the objective tumor response according to RECIST v1.1 guidelines.[1] Additional assessment of anti-tumor activity will be explored based on total measurable tumor burden.[39] (Appendix 4)

Pharmacodynamic Evaluations	Immunological and biological markers of response will include examinations of tumor biopsy samples, cytokine levels, peripheral blood mononuclear cells (PBMC) and antibody responses.
Pharmacokinetic Evaluations	INXN-1001 pharmacokinetics will be evaluated in all subjects during Cycle 1 only.
Sample Size Determination	No formal sample-size estimation was performed. The choice of the number of subjects was based on the standard Phase I 3+3 inter-cohort dose escalation design in which 3 to 6 subjects are enrolled into each dose level cohort. In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. Based on a review of available safety and clinical response data, the SRC may identify a single dose cohort to be expanded to a total of 15 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving 3 cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis. Given these considerations, a total of approximately 30 subjects may be enrolled into this study, including 3 subjects enrolled in each of the 4 planned dose level cohorts and approximately 15 additional subjects enrolled at or below the MTD during the Phase II portion of the study. Additional subjects may also be enrolled as replacement subjects or to explore intermediate dose level cohorts.

4. TABLE 2. SCHEDULE OF ASSESSMENTS

Activity	FOR ALL PHASE II GROUP 2 SUBJECTS									
	Screening[1]		ALL Cycles						Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Day 1	Day 2	Day 3 OR Day 4[27]	Day 7	Day 13	Day 15	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Clinical Assessments										
Informed Consent[2]	X									
Medical/Cancer History[3]	X									X[30]
Physical Exam[4]	X	X	X				X		X	
ECOG PS[5]	X		X						X	
Height	X									
Weight	X	X	X						X	
Vital Signs[6]	X	X	X	X	X[28]	X	X	X	X	
Adverse Events[7]	X	→	→	→	→	→	→	→	X	
Concomitant Med.[8]	X	→	→	→	→	→	→	→	→	X
Clinical Laboratory										
Pregnancy test[9]	X		X							
Hematology tests[10]	X		X[22]			X		X	X	
Serum Chemistry tests[11]	X		X[22]			X		X	X	
Urinalysis[12]	X		X[22]					X	X	
ECG[13]	X		X						X	
Serum Tryptase[14]			←	→	X	←	→			
Subject Registration[15]	X									
Study Drug Administration										
Intratumoral INXN-2001[23]			X							
Oral INXN-1001[24]			←	→	X	←	→			
Verify Adherence to INXN-1001 dosing[25]								X		

Activity	FOR ALL PHASE II GROUP 2 SUBJECTS									
	Screening[1]		Cycles 1, 3, and 6 ONLY						Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Day 1	Day 2	Day 3 OR Day 4[27]	Day 7	Day 13	Day 15	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Ab responses[16]		X						X	X	
Cytokine profile[17]		X			X			X	X	
INXN-1001 PK[26] CYCLE 1 ONLY			X	X			X			

Activity	FOR ALL PHASE II GROUP 2 SUBJECTS						
	Screening[1]		Cycles 1, 3, and 6 ONLY			Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Cycle 1 Day 13±2	Cycle 3 Day 13±2	Cycle 6 Day 13±2	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Tumor Response / Pharmacodynamics							
Imaging Studies/Tumor Assessment[18]	X			X		X	X
Digital photography[19]	X		X		X	X	X
PBMC[20]		X		X		X	X
Biopsy [21]							
Tumor injected with INXN-2001				X			
Non-Injected Tumor and/or Draining Lymph Node		X		X			

Table 2 Footnotes:

1. All screening assessments will be performed within 28 days prior to the first dose of study drug.
2. Written informed consent must be signed by the subject before any protocol required procedures and assessments are performed. Standard of care evaluations that were performed as part of the subject's routine treatment prior to signing consent can be used if they were conducted in the timeframe allowed for screening.
3. Medical history includes demographic information and medical and surgical history. Cancer history includes current cancer diagnosis, treatment regimens (regimen, doses, start and stop dates), and best response for each regimen.
4. A complete physical exam is required at the Screening and the Post-Treatment Safety Assessment visits. Otherwise, a symptom-directed physical exam should be performed where indicated.
5. ECOG (Eastern Cooperative Oncology Group) performance status (Appendix 1).
6. Vital signs include blood pressure, pulse, temperature, and respirations. On Day 1 of each cycle, vital signs will be recorded prior to INXN-1001 dosing and hourly for the first two hours after INXN-2001 dosing. Blood pressure is to be monitored closely to prevent hypotension after administration of INXN-1001. Blood pressure assessment is required on Day 3 or Day 4 for Cycle 1 and Cycle 2. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted]
7. Monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. AEs/SAEs that occur following informed consent until the Post-Treatment Safety Assessment must be recorded on the AE CRF. In addition, all SAEs must be reported by the investigator or designee within 24 hours of becoming aware of the event, from the time of informed consent through 30 days after the last dose of study drug, regardless of the initiation of any new anti-cancer therapy.

8. Concomitant medication information, including blood products, vitamins and other supplements will be collected for the time period beginning 28 days prior to the first dose of study drug, through the Follow-Up Tumor Assessment visit.
9. Females of childbearing potential will have a serum pregnancy test at the Screening visit and a urine pregnancy test on the first day of each treatment cycle, prior to administration of INXN-1001.
10. Hematology tests include: complete blood count and white blood cell count, differential white blood cell count, red blood cell count, hematocrit, hemoglobin, red blood cell indices, reticulocyte count, MCV (mean corpuscular volume) and platelet count. PTT (partial thromboplastin time) and INR (international normalized ratio) will also be evaluated.
11. Serum chemistry tests include: aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, blood urea nitrogen, glucose, sodium, potassium, chloride, calcium, phosphorus and bicarbonate.
12. Urinalysis panel (dipstick) includes: appearance, pH, specific gravity, glucose, protein/albumin, blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated.
13. Standard, single 12-lead ECG at screening, 2½ to 3 hours after INXN-1001 dosing (prior to INXN-2001 injection) on Day 1 of each cycle, and at the Post-Treatment Safety Assessment visit.
14. Serum tryptase level will be obtained upon the occurrence of a suspected injection and/or hypersensitivity reaction. Otherwise, this test is not required at any other time.
15. For subjects who meet inclusion and exclusion criteria. Centralized registration of eligible subjects will be completed according to a process defined by the sponsor.
16. Blood sample for analysis of antibody responses to the adenovirus and/or the RTS[redacted] components, and antibodies to melanoma-associated antigens. Please refer to the laboratory manual for details regarding sample processing and shipment.
17. Blood samples for cytokine profiling. Please refer to the laboratory manual for details regarding sample processing and shipment.
18. Appropriate cancer staging procedures should be performed during screening. For the purpose of this clinical trial, the following imaging is expected at screening:
    a. CT of the chest, and CT (or MRI) of the abdomen and pelvis
    b. MRI (or CT) of the brain if brain metastasis are known or suspected
    c. CT or MRI of other anatomical regions as clinically indicated

    For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable).

    Disease sites are to be assessed throughout the study using the same method(s) used at screening. Specific image acquisition guidelines will be provided by a central imaging laboratory. Chest, abdomen, pelvis imaging is required for all follow-up imaging time points; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated.

    NOTE: As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, anti-tumor response will be explored by measuring total tumor burden over time. (see Appendix 4)

19. Digital photograph(s) of all visible injected tumor(s) and non-injected tumors, and of any visible local reactions in or around the injected lesion(s). Photographs are required at screening, Cycle 1 Day 13, Cycle 6 Day 13, PTSA and FUTA visits and ad hoc as the investigator deems necessary. Any subject who has or develops vitiligo should be documented the same way. Details regarding photographic methodology will be provided in the Photography Operations Manual.
20. For Phase II: blood samples for cellular immune response (PBMC) analyses are to be collected at Screening, Cycle 3 Day 13, the Post-Treatment Safety Assessment, and Follow-Up Tumor Assessment visits. Please refer to the laboratory manual for details regarding sample processing and shipment. **NOTE:** If a subject is off-study before Cycle 3, then a blood sample will be obtained at the PTSA visit.
21. Punch biopsies (PBx) of tumor(s) and/or associated tumor involved draining lymph nodes will be collected for *in vivo* assessment of transgene function and immunological activities, and other biological effects should be obtained at the time points indicated in the preceding table. Details of these procedures are described in the laboratory manual. **NOTE:** If a subject is off-study before Cycle 3, then a PBx will be obtained at the PTSA visit.

    PBx: Punch biopsy (≥4 mm in diameter)

    - At screening Day -6 to -2, punch biopsies should be obtained only after subject registration has been completed (ie, after approval by sponsor for study participation) and before start of treatment.
22. Day 1 clinical laboratory assessments may be performed within 2 days prior to the Day 1 clinic visit. Clinical laboratory tests drawn for analysis on Day 15 of each cycle may be used for determining if the retreatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration (see Section 8.2.6).
23. Intratumoral INXN-2001 [redacted] injection should be given 3 hours ± 30 minutes after the INXN-1001 dose. Lesions displaying signs of local infection should not be injected. Subjects must be adequately hydrated on each day of study drug administration, and must be instructed to maintain good oral hydration on and between dosing days. Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions, for at least 2 hours following the INXN-2001 injection. A detailed description of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions will be documented. The subject should be instructed to call the clinical site if any such reactions develop or don't resolve within 24 to 48 hours. Please refer to Section 8.2.5 for additional information.
24. The first INXN-1001 dose will be given on Day 1 at the clinical site in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal and then the remaining 6 doses will be self-administered every other day at the same time (±1 hour) in the fed state. Subjects must be adequately hydrated on each day of study drug administration, and must be instructed to maintain good oral hydration on and between dosing days. [redacted] Study sites must verify compliance with INXN-1001 dosing on Days 5, 11, and 13. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day 15 visit of each cycle. Please refer to Section 8.2.5 for additional information.
25. Review and reconcile returned treatment container(s) and remaining capsules to assess the degree of adherence. All subjects will be queried about dose schedule adherence and reasons for missed doses.

26. FOR CYCLE 1 ONLY: Subjects will undergo an INXN-1001 pharmacokinetic (PK) assessment. Blood samples for INXN-1001 PK analysis should be obtained on the following days and time points:

    Day 1: Pre-dose (< 30 minutes prior to INXN-1001 dosing)
    1-2 hours post INXN-1001 dosing
    3-6 hours post INXN-1001 dosing
    Day 2: Pre-dose (< 30 minutes prior to INXN-1001 dosing)
    Day 13: 1-2 hours post INXN-1001 dosing
    3-6 hours post INXN-1001 dosing
    Day 15: Anytime >24 hours post-Day 13 INXN-1001 dose

27. Blood pressure will be assessed at Cycle 1 and Cycle 2 Day 3 or 4 visit. During Cycle 1 and 2, Day 3 or 4 visits are required. For Cycles 3-6, Day 3 or 4 visits should be performed when feasible (eg, Monday through Friday).
28. Post-Treatment Safety Assessment visit will be performed 28 ± 3 days after the last dose of INXN-1001 or at the time of early withdrawal from study treatment.
29. A Follow-Up Tumor Assessment visit will be performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. This assessment is required for all subjects, including those with prior objective evidence of disease progression to ensure that more slowly declining tumor burden in response to therapy is not missed.

    Subjects without objective evidence of disease progression should continue to have tumor assessments performed at 8 -10 weeks intervals until irPD has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.
30. Interim cancer history information will include documentation of any new concomitant medications and any anti-cancer treatments received since the Post-Treatment Safety Assessment visit or previous tumor assessment.

5. INTRODUCTION

5.1 DISEASE BACKGROUND (MELANOMA)

The incidence of melanoma has been increasing steadily in the U.S. and other developed countries for decades. At present, the incidence rate among the U.S. population is roughly 1 in 60. Solar radiation exposure is an important risk factor, but genetic risk factors multiply this, and a family history of melanoma is a strong indicator of risk.

Current treatment of melanoma involves surgical resection, which is highly effective when the melanoma is identified early. Systemic chemotherapy with a number of standard anti-neoplastic agents may have transient palliative success for more advanced disease that is beyond surgical cure, but there is no evidence of any significant prolongation of survival with any systemic currently available agents.[2] Immunotherapy approaches have been employed with evidence of significant benefit and include the use of interferon (IFN) alpha-2b, interleukin-2 (IL-2) and the experimental administration of interleukin-12 (IL-12), as well as tumor necrosis factor (TNF)-alpha and other biological agents. High dose IFN alfa-2b (INTRON® A) has demonstrated an improvement in survival for Stage IIB- III patients in the adjuvant (postoperative) setting. High dose IL-2 (Proleukin®) has been approved by the FDA for treatment of patients with metastatic (Stage IV) melanoma on the basis of Phase II trials showing prolonged survival in a subset of patients (16%) who demonstrate tumor regression with this agent; however, the side effects of systemically administered IL-2 and IFN alfa-2b can be severe and limiting. Ipilimumab, an anti-CTLA-4 monoclonal antibody used to overcome or reverse T cell suppression and thereby potentiate an anti-tumor T cell response, was approved by the FDA for treatment of unresectable Stage III and IV melanoma on the basis of a Phase III trial which demonstrated an improvement in median overall survival. In that study, there were 14 deaths related to the study drugs and 7 were associated with immune related adverse events.[3] Other immunotherapies for the treatment of melanoma are also in various stages of development, including immune checkpoint inhibitors, and OncoVEX^{GM-CSF}, an oncolytic herpes simplex virus encoding GM-CSF.

The results from these studies demonstrate that other, safer and still effective immunotherapies are greatly needed for patients with advanced melanoma.

5.2 BACKGROUND INFORMATION FOR GENE THERAPY COMPONENTS

[redacted]

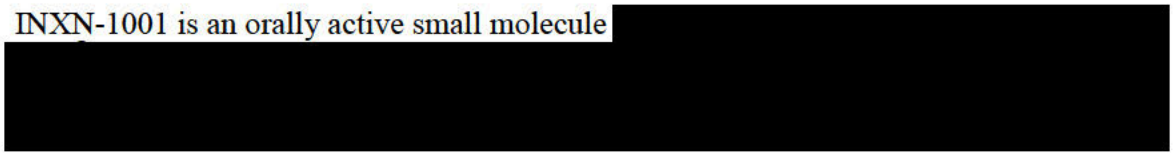
The Phase I/II study proposed in this protocol (ATI001-101) will examine the safety, anti-tumor activity, and immunological effects of intratumoral injections of INXN-2001 in combination with orally administered INXN-1001 as a potential anticancer therapy in subjects with advanced melanoma.

5.2.2 INXN-2001 (Ad-RTS-hIL-12)

INXN-2001 (Ad-RTS-hIL-12) is comprised of the replication-incompetent adenovirus vector with human interleukin-12 (hIL-12) as the [redacted] regulated transgene (Figure 2), as described by Anderson *et al*.[6] Cells transduced with INXN-2001 will produce hIL-12 protein if transcription has been activated in the presence of the small molecule ligand, INXN-1001. Because the level of transgene expression (ie, protein production) is ligand-concentration dependent, hIL-12 production can be modulated by the dose and frequency of oral INXN-1001 administration. Therefore, potential IL-12-related adverse events may be relieved by withdrawal of INXN-1001 administration, if necessary.

5.2.3 INXN-1001 (ACTIVATOR LIGAND)

INXN-1001 is an orally active small molecule



5.3 BACKGROUND AND RATIONALE FOR TREATMENT STRATEGY

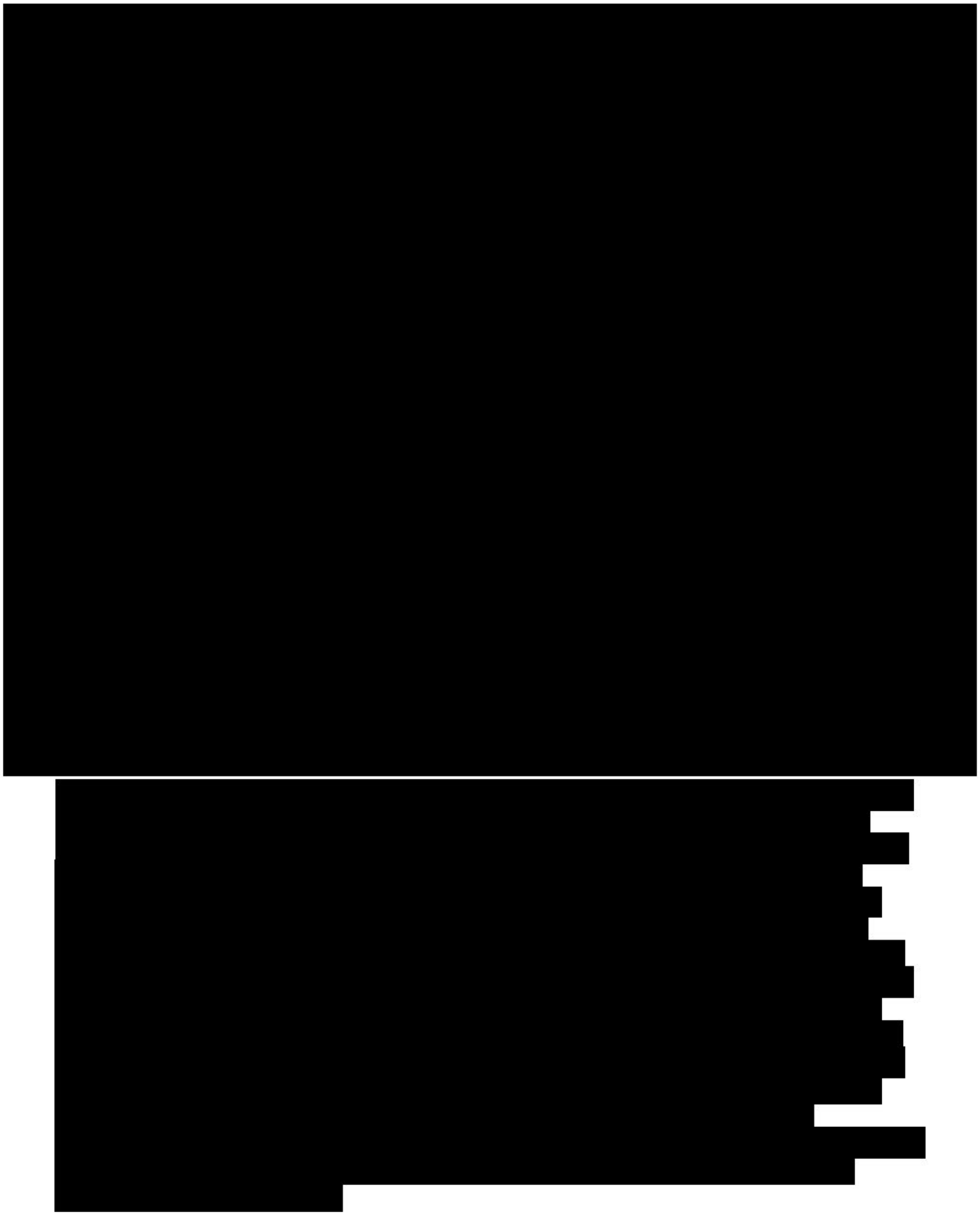
5.3.1 IL-12 Immunotherapy

Cytokine-mediated, anti-tumor effects have been studied in a number of tumor model settings as well as clinical trials. The short half-lives of most cytokines, coupled with the cost and difficulties of manufacturing, delivery, and severe side effects associated with high systemic doses, have limited their use as a therapy. Although small amounts of endogenously synthesized cytokines may circulate systemically, cytokines are generally produced and act at precise sites in very high local concentrations. These facts have made attractive the local delivery of cytokines directly into tumors. This might be accomplished by gene therapy to enhance the local anti-tumor response and locally reverse tumor-induced tolerance. Indeed, local cytokine delivery to tumors can induce a number of anti-tumor responses that include: 1) direct suppression of tumor cell proliferation, 2) induction of apoptosis, 3) anti-angiogenesis, and 4) enhancement of local anti-tumor immune response. This enhancement of local anti-tumor response can be further characterized by

cytokine-mediated activation of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), as well as the modulation of major histocompatability molecule expression.[8]

Interleukin (IL)-12 is a potent immunostimulatory cytokine which has promise as a cancer therapeutic agent that remains to be realized in the clinic. IL-12 activates and recruits dendritic cells (DC) which in turn, facilitate the cross-priming of tumor antigen-specific T cells. IL-12 functions as a key mediator for the generation of TH1 CD4+ effector T cells, activation of natural killer (NK) and $CD8^+$ T cell cytotoxic activities, and the production of IFN-γ and TNF-α.[9-12] IL-12 also has anti-angiogenic activity. Administration of IL-12 has been shown to significantly reduce or eradicate tumor growth and to enhance survival in numerous animal models of cancer.[13-17] Significant results observed in these tumor models after IL-12 treatment have been correlated with the ability of IL-12 to stimulate anti-tumor immunity and suggests that this cytokine may be applied as a novel immunotherapy in human cancers.

Clinical trials have been performed to assess the safety and efficacy of administering recombinant IL-12 protein to subjects with cancer, including, but not limited to, melanoma, renal cell carcinoma, colorectal, cervical, ovarian, and lung metastases. IL-12 protein was delivered via intravenous (IV), intrapertitoneal (IP), or subcutaneous (SC) administration.[18-27] Administration of IL-12 systemically resulted in induction of immune responses in some subjects but there was limited clinical improvement and severe toxicity. In a Phase II trial evaluating repeated iv administration of rhIL-12 in subjects with renal cell carcinoma, 12/17 subjects were hospitalized and two died due to hypovolemic shock. The study was halted as most subjects experienced SAEs during the first cycle of treatment. There was a high frequency of Grade 3 and 4 AEs, with 65% of subjects showing leukopenia and 47% showing hyperbilirubinemia and elevated AST.[27] Based on reports to date, strategies for the local delivery of IL-12 through gene therapies may represent a safer and acceptable treatment approach in cancer patients.



[redacted]

5.4 SUMMARY OF PRIOR CLINICAL EXPERIENCE AND SAFETY

5.4.1 INXN-1001 and INXN-2001

No clinical studies of INXN-1001 in combination with INXN-2001 have been previously performed. [redacted]

Please refer to the current Investigator's Brochure for additional information regarding the previous nonclinical and clinical experience with INXN-1001 and INXN-2001.

[redacted]

5.4.2 Adenovirus Safety

Adenoviral vectors have been used extensively to deliver a variety of gene products to human subjects including cancer patients. Although adenoviral vectors are immunogenic, and virtually all recipients have pre-existing humoral immunity to adenoviruses, they are generally considered to be a safe, well tolerated method of gene delivery that results in minimal side effects in patients.

In a Phase I/II clinical trial in prostate cancer (PCa), direct intraprostatic injection of a replication-defective adenovirus vector encoding bacterial nitroreductase (dose levels 5 x 10^{10}-1 x 10^{12} virus particles), the vector was well tolerated, with minimal side effects, and had a short half-life in the circulation.[28] Numerous studies have utilized adenoviral vectors to deliver the p53 gene via the intratumoral route. Oral leukoplakia patients who were given multiple intraepithelial injections of recombinant adenovirus (rAd)-p53 (1 x 10^{8} virus particles/cm^2) did not demonstrate signs of dose-limiting toxicity, and administration of the (rAd)-p53 was well tolerated.[29] No dose-limiting toxicity was observed in chemoradiation-resistant advanced esophageal carcinoma patients receiving intratumoral injections of adenovirus containing p53 (10 x 10^{11} particles to 25 x 10^{11} particles).[30] Adverse events attributed to Ad.5CMV-p53 treatment were generally mild to moderate, with the most common AEs being Grade 1 or 2 fever (100 % of patients) and local pain (30% of patients.) Three patients displayed hyperglycemia that was attributed to nutrition, two patients showed hypocalcemia, and one patient each experienced partial thromboplastin time elongation or an increase in serum amylase or creatinine. No other significant laboratory abnormalities were detected on follow-up evaluations.[30]

An adenoviral vector encoding hIL-12 has been studied using intratumoral injection in patients with advanced digestive malignancies, at doses up to 3 x 10^{12} viral particles, and was well tolerated. Common AEs were similar to symptoms observed with gene delivery by other adenoviral vectors, including transient, mild to moderate fever, malaise, sweating, and lymphopenia.[31]

5.4.3 Safety of Intratumoral injection of IL-12 genes

In comparison with administration of recombinant IL-12 protein, injection of plasmids or adenovirus containing hIL-12 genes to cancer patients has proven to limit or abrogate the toxic effects of hIL-12, thus providing an effective way to provide this potent immunomodulatory cytokine. This has been observed in Phase I and II clinical trials where IL-12 is delivered locally, IL-12 levels have been measured systemically in patient serum, and in the majority of these studies, increases in IL-12 serum levels post baseline have not been observed.[31-33] Numerous studies have shown gene delivery of IL-12 to be well tolerated. In a Phase I dose escalation trial using electroporation to administer plasmid DNA encoding hIL-12 into metastatic melanoma, minimal systemic toxicity and no IL-12-related adverse events were observed in subjects treated at several dose levels.[34] Similarly, intratumoral injection of a plasmid DNA encoding human IL-12 (2 to 20 mg of total DNA) in subjects with melanoma was also well tolerated.[35] Limited elevations in serum cytokines were observed after treatment. Intra-peritoneal injection of hIL-12 plasmid DNA formulated in polyethyleneglycol-polyethyleneimine-cholesterol (PPC) given to women with chemotherapy-resistant recurrent ovarian cancer also was found to be generally safe and well tolerated. Low-grade fever and abdominal pain were the most common side effects.[36] Plasmid DNA was not detected in the patients' serum samples and treatment-related increases in IFN-γ levels were observed only in pleural fluid but not in serum. Similar findings have been obtained with intratumoral injection of adenoviral vectors encoding hIL-12 at doses ranging from 2.5×10^{10} to 3×10^{12} viral particles, to subjects with advanced pancreatic, colorectal, or primary liver malignancies.[31] Treatment was well tolerated and dose-limiting toxicity was not reached. AEs were transient, with fever, malaise, sweating, and lymphopenia being the most common AEs observed. No cumulative toxicity was observed. All AEs were deemed to be related to injection of the virus and not to transgene expression.

5.5 RATIONALE FOR STUDY DESIGN AND DOSE SELECTION

The objectives of this Phase I/II clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study (Phase I) uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, there will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. Phase II will be enrolled (approximately 15 subjects) at or below the MTD and using two groups. One group will receive an INXN-1001 daily for 7 days dosing regimen, and the other group will receive an every other day dosing regimen for 14 days to compare tolerability of INXN-1001 at the 160-mg dose.

Phase I: In each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001.

Phase II, Group 1: In each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001.

Phase II, Group 2: In each of six 28-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001 administered every other day for 14 days.

The INXN-1001 dose levels to be studied (5 to 160 mg/day) are within the range of doses administered in previous human clinical trials [redacted] and are expected to provide plasma levels that are biologically effective for [redacted] activation. Although good safety and tolerability is expected based on previous clinical experience, rigorous safety monitoring during the course of this study will be performed by the Safety Review Committee (SRC).

In all treatment cycles throughout the study, the INXN-2001 dose will be fixed [redacted] dose. This dose was selected based on: (i) preclinical studies that indicated that this would be within an effective range, and (ii) previous clinical trials of administered adenoviral vectors [redacted]

During the conduct of this study, in addition to monitoring of safety and therapeutic efficacy, tumor tissue and peripheral blood samples will be studied for indications of immunological and biological effects, with a particular attention to markers of IL-12 gene expression and levels of specific anti-melanoma T cell immunity. The combined clinical and laboratory data will be studied to assess for INXN-1001 dose-response relationships and to aid in identifying a recommended dose level for further study.

The population for study was selected as those with unresectable Stage III or IV melanoma, which is rarely curable with standard therapy and who are therefore considered to be appropriate candidates for clinical trials exploring new forms of treatment.[37] Because melanomas are responsive to immunotherapeutic approaches (see Section 5.1 Disease Background), this subject population may potentially benefit from investigational therapy with INXN-2001.

6. STUDY OBJECTIVES AND DESIGN

6.1 OBJECTIVES

The primary objective is to:

- Evaluate the safety and tolerability of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) in combination with INXN-1001 (activator ligand) in subjects with unresectable Stage III or IV melanoma.

The secondary objectives are to:

- Inform the selection of an INXN-1001 dose(s) and regimen for further study in combination with INXN-2001.
- Assess preliminary anti-tumor activity according to RECIST 1.1 criteria.

- Assess anti-tumor response based on total measurable tumor burden.
- Evaluate the immunological effect of study treatment in terms of cellular and humoral immune responses, as well as other biological activities in the injected tumor(s), tumor-involved draining lymph nodes (if accessible) and in the peripheral circulation.
- Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells, and to assess adenoviral vector shedding in body fluids.
- Assess the pharmacokinetics (PK) of INXN-1001 in subjects with unresectable stage III or IV melanoma.

6.2 STUDY DESIGN AND DOSE ESCALATION PROCEDURE

6.2.1 Overall Study Design

This is a single-arm, open label, Phase I/II dose escalation study of intratumoral injections of INXN-2001 and oral INXN-1001 in subjects with unresectable Stage III or IV melanoma.

Phase I: Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to 6 treatment cycles, each 21 days in duration. Previously enrolled subjects received 3 cycles under former versions of this protocol. There will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. No intrasubject dose escalation will be allowed.

Phase II: Approximately 15 subjects will be enrolled in Phase II at or below the MTD. For Group 1, in each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. For Group 2, in each of six 28-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001 administered every other day for 14 days.

Safety and tolerability will be assessed by the incidence and severity of adverse events as determined by NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03. A SRC comprised of the Medical Monitor, Principal Investigators, and sponsor representatives, will be convened to review safety information and to decide upon dose escalation and further subject enrollment.

The anti-tumor activity of study treatment will be assessed according to RECIST v1.1 guidelines. Additional assessment of anti-tumor activity will be explored based on total measurable tumor burden.[39] (Appendix 4)

Immunological and biological markers of response will include examinations of tumor biopsy samples, cytokine levels, PBMCs and antibody response to INXN-2001.

6.2.2 Treatment Parameters and Duration

All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of each cycle. The subjects will also receive an oral daily dose of INXN-1001 for 7 consecutive days (Phase I and Phase II Group 1) or every other day for 14 days (Phase II Group 2) starting on Day 1 of each cycle.

Subjects may receive up to 6 cycles of study treatment. Because tumor nodules may enlarge due to the anticipated induction of an inflammatory response, subjects with disease progression (per RECIST criteria) may still complete the 6 treatment cycles, unless the progression is clinically significant, eg, development of new metastases or an ECOG performance status that increases during study treatment to ≥ 2.

6.2.3 Safety Review Committee (SRC)

A SRC comprised of the Medical Monitor, Principal Investigators and sponsor representatives, will hold periodic teleconferences to evaluate the safety and treatment status of all subjects. The SRC will review and assess the safety data at the completion of each dose cohort as described in Section 6.2.6, and at any other time as needed. The SRC has the authority to recommend dose modifications for safety concerns.

A written summary documenting the results and recommendations of each review will be provided to the Investigator(s) and maintained on file with the sponsor. Additional sub-Investigators and personnel may participate in reviews as indicated.

In Phase I, following a cohort review, the SRC may recommend proceeding with enrollment in the next dose cohort, enrolling additional subjects in the current cohort, dropping back to a lower cohort, exploring an alternate dose level, or not enrolling any additional subjects. The dose escalation and enrollment guidelines outlined in Section 6.2.6, as well as the study stopping rules outlined in Section 6.2.7, will be used as the basis for these assessments.

In Phase II, Group 2, the DLT definitions in Section 6.2.4 will be utilized along with the criteria for de-escalation. The SRC may recommend a dose reduction for subjects in Group 2 if one or more DLT(s) are observed.

While the SRC is expected to reach a consensus opinion regarding any premature discontinuation or significant modification of the study, the sponsor may independently stop the study.

6.2.4 Definition and Management of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) is defined as:

- Any injection site reaction that includes ulceration, necrosis, severe tissue damage or where operative intervention is required.
- Any local or systemic injection reaction that has life-threatening consequences, requires urgent intervention or results in death.

- Any study drug-related ≥Grade 3 adverse event, except nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics.

Phase I and Phase II, Group 1: If DLTs have been observed in >1 of the 3-6 subjects in a cohort during the first treatment cycle, the MTD will have been exceeded and further use of that dose will be discontinued in all subjects, and the next lower dose cohort will be assessed as to whether it meets the definition of the MTD.

Phase II, Group 2: If an adverse event that meets the Phase I DLT definition is observed in >1 of the 3-6 subjects in Group 2 during the first treatment cycle, the SRC will convene to discuss reduction in dose and/or change to posology and the cohort will be expanded by 3 subjects. The recommended Phase II dose will be the dosage and posology where ≤1 of 3 to 6 subjects experiences an adverse event as described in the definition of DLT.

The Medical Monitor should be immediately notified of any potential DLT. Subjects will be withdrawn from study treatment if they experience an injection site reaction that meets DLT criteria or other Grade 3 toxicity related to study drug that, upon review of the SRC, is deemed to be a DLT. If, however, the subject has experienced a substantial benefit from treatment and the SRC recommends continuation on the study, re-exposure to the experimental treatment may proceed at a lower dose of INXN-1001 on a case by case basis. Any subject who experiences a Grade 4 toxicity related to study treatment will be withdrawn from study.

6.2.5 Definition of Maximum Tolerated Dose (MTD) and Recommended Phase II Dose (RP2D)

The MTD will be defined as the highest dose of INXN-1001 studied in combination with INXN-2001 at which a DLT has been seen in a maximum of 1 in 6 subjects (< 33%) during the first treatment cycle. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered.

The recommended Phase II dose will be the dosage and posology where ≤1 of 3 to 6 subjects experiences an adverse event as described in the definition of DLT.

6.2.6 Dose Escalation Procedure

In Phase I, four sequential INXN-1001 dose escalation cohorts are planned, as described in Appendix 5. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. No intrasubject dose escalation will be allowed. An intermediate dose level(s) of INXN-1001 may be explored after review of all available safety and activity data, as may be decided by the SRC.

Approximately 15 subjects will be enrolled in Phase II at or below the MTD

To increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed.

6.2.7 Study Stopping Rules

Rules for stopping dose escalation due to DLTs observed during the first cycle of study treatment are outlined in Section 6.2.4. In the event that a given dose is determined to have exceeded the MTD, then further use of that dose will be discontinued for all subjects as noted in Section 6.2.4. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered.

Stopping rules for toxicities that occur from Day 1, Cycle 2 through the Post-treatment follow-up visit will apply as follows:

- If an adverse event can be definitely shown to be unrelated to study treatment (eg, motor vehicle accident, bee sting, elective surgery), that specific toxicity will not be considered in a stop vs. dose modification decision.
- Recruitment in the current dose level will not be stopped for Grade 2 (NCI CTCAE v.4.03) non-dose limiting toxicities, irrespective of the number of events or number of subjects.
- Recruitment in the current dose level will be stopped for any Grade 4 toxicity. Upon occurrence, all data will be reviewed by the Investigator, the Investigator's IRB as applicable, ZIOPHARM Medical and Regulatory Staff, and the SRC to determine if the study should proceed and if/what changes must be implemented.
    - For toxicity based on laboratory tests: an abnormality related to study drug per investigator assessment must be confirmed (when appropriate) by repeat testing before qualifiying for review as above.
- Subject enrollment into the study will be halted in the event of any study drug-related death. In this case, subject enrollment will only resume following review and approval of the SRC.

7. SUBJECT SELECTION

The target population for this study is adult subjects with unresectable Stage III or IV melanoma for which there is no alternative curative therapy.

7.1 INCLUSION CRITERIA

To be enrolled in the trial, each subject must satisfy all of the following inclusion criteria:

1. Males or females of all races ≥ 18 years of age, who have provided written informed consent prior to completing any study specific procedure.
2. Unresectable Stage III or Stage IV melanoma arising from any site other than ocular melanoma.
3. A minimum of 2 accessible lesions (shortest diameter ≥1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥1.5 cm).

4. ECOG performance status of 0 or 1 (Appendix 1).
5. Adequate bone marrow, liver, and renal function, as assessed by the following laboratory requirements:
    a. Hemoglobin ≥ 9 g/L
    b. Lymphocytes > 700/ mm^3
    c. Neutrophils ≥ 1,500/ mm^3
    d. Platelets ≥ 100,000/ mm^3
    e. Serum creatinine ≤ 1.5 x ULN
    f. AST and ALT ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5×ULN
    g. Total bilirubin < 1.5 x ULN
    h. International Normalized Ratio (INR) and activated partial thromboplastin time [PTT] <1.5 x ULN, if not therapeutically anticoagulated. Subjects who are being therapeutically anticoagulated with an agent such as Coumadin (warfarin sodium) or subcutaneous heparin may be included provided there is no prior evidence of underlying abnormality in coagulation parameters, screening test results are in appropriate therapeutic range, and anticoagulation regimen is stable and closely monitored.
6. An expected survival of at least 6 months.
7. Male and female subjects must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the screening visit through 28 days after the last dose of study drug.

7.2 EXCLUSION CRITERIA

Subjects will be excluded from enrolling in the trial if they meet any of the following exclusion criteria:

1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug.

    NOTE: For Phase II ONLY, if subjects received ipilimumab, a 45-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will be made.
2. Clinically significant infection requiring systemic antibacterial, antifungal, or antiviral therapy within 2 weeks of the first dose of study drug.
3. History of HIV infection.
4. Active autoimmune disease requiring steroids (>10 mg prednisone or comparable) or other immunosuppressive therapy (eg, methotrexate, etc.).

5. Documented symptomatic brain metastases. Screening for brain lesions by CT or MRI is not required for all potential subjects; however, if there are any neurological signs or symptoms consistent with brain metastases, then a brain CT or MRI should be performed as clinically indicated.
6. Any medications that induce, inhibit or are substrates of CYP450 3A4 within 7 days prior to the first dose of study drug.
7. Prior history of hematopoietic stem cell transplant or organ allograft.
8. Other concurrent clinically active malignant disease, with the exception of other cancers of the skin.
9. Females who are nursing or pregnant.
10. Subjects who have a history of hypersensitivity that may relate to any component of the study drugs, eg to benzoic acid since INXN-1001 contains two benzene rings.
11. Unstable or clinically significant concurrent medical condition that would, in the opinion of the investigator, jeopardize the safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, unstable angina, congestive heart failure, recent (within 2 months of screening) myocardial infarction, ongoing maintenance therapy for life-threatening ventricular arrhythmia, uncontrolled asthma, evidence of hepatic pathology due to or consistent with infection with a chronic hepatitis virus, uncontrolled major seizure disorder, or electrolyte imbalances.
12. Local infection at site of injectable lesion requiring anti-infective therapy within 2 weeks of the first dose of study drug.

7.3 WITHDRAWAL OF SUBJECTS FROM STUDY TREATMENT AND/OR STUDY

The investigator and/or the subject have the right to terminate the subject's participation in the study at any time. Subjects who discontinue study drug should complete the Post-Treatment Safety Assessment visit.

A subject **may** withdraw (or be withdrawn) from the study treatment prematurely for any of the following reasons:

- Principal investigator (PI) determines further participation is not in subject's best interest.
- Clinically significant disease progression.
  NOTE: In the absence of rapid clinical deterioration, disease progression should be confirmed by a repeat, consecutive assessment no less than 4 weeks from the date first documented to ensure that more slowly declining tumor burden in response to therapy is not missed.

A subject **MUST** be withdrawn in the event of any of the following:

- Withdrawal of informed consent. (Note: Any subject who wishes to withdraw from the study treatment may do so but will be counseled that follow-up for the effects of immunotherapy is strongly recommended.)
- Any DLT meeting the withdrawal criteria described in Section 6.2.4.
- Any treatment-related adverse event meeting withdrawal criteria as described in Section 8.2.6.
- Substantial noncompliance with the requirements of the study.
- Subjects with a confirmed positive pregnancy test (to be reported via pregnancy notification form).
- Any intercurrent illness that would, in the judgement of the investigator, affect assessments of clinical status to a significant degree and require discontinuation of protocol therapy.

Every effort should be made to follow subjects who withdraw from study treatment with ongoing treatment-related adverse events in order to determine the outcome of the event.

7.4 REPLACEMENT OF SUBJECTS

Subjects who are withdrawn from study treatment prior to completing Cycle 1 dosing for reasons other than toxicity may be replaced so that a full cohort of subjects completes Cycle 1 safety evaluations. In addition, should a subject discontinue study treatment before receiving 3 cycles for reasons other than toxicity, another subject may be added to that dose cohort so as to result in a total of at least three subjects for full analysis.

7.5 PREMATURE TERMINATION OF STUDY/CLOSURE OF CENTER

The sponsor has the right to close the study at any time, although this should occur only after mutual consultation between the sponsor and the investigators. The IRB/IEC must be informed. Should the study/center be closed prematurely, all study materials (completed, partially completed, and blank case report forms, study medication, etc) must be stored or disposed of according to the sponsor's instructions. Events that may trigger premature termination of the study or closure of a center include, but are not limited to a new toxicity finding, results of any interim analysis, noncompliance with the protocol, change in development plans for the study drug, slow recruitment, or poor-quality data.

8. TREATMENT PROCEDURES

8.1 SCREENING

Principal investigators (PIs) at each site are responsible for maintaining a record of all subjects screened, including both those who enter the study and those who are excluded.

8.1.1 Informed Consent

Each potential subject must sign a written, informed consent form (ICF) prior to performing any study specific screening procedure.

8.1.2 Inclusion and Exclusion Criteria

Inclusion and exclusion criteria will be reviewed for each potential subject and documented in the CRF.

8.1.3 Registration of Subjects

Centralized registration of subjects will be completed according to a process defined by the sponsor. The sponsor will assign a subject specific identification number and note the dose cohort to which the subject is assigned. Once assigned, a subject's identification number will not be reused.

8.2 STUDY DRUG

8.2.1 Accountability and Dispensation

The Principal Investigator (PI) must maintain accurate records accounting for the receipt and dispensation of the study drugs.

The investigational materials are to be prescribed only by the PI or the sub-investigators named on the Form FDA 1572, and may only be dispensed by authorized personnel at the institution(s) listed therein.

Under no circumstances will the PI allow investigational drug to be used other than as directed by the protocol.

8.2.2 Handling and Storage

Study drugs must be stored in a restricted access area under the storage conditions indicated on the product label or Investigator's Brochure.

All necessary precautions while handling potentially toxic compounds must be strictly followed.

8.2.3 Treatment Regimen

Each subject's assigned INXN-1001 dose will be given orally daily for the first 7 consecutive days of each 21-day cycle (Phase I and Phase II Group 1). For Phase II Group 2 the INXN-1001 dose will be given orally every other day for 14 days of each 28-day cycle,

INXN-2001 will be given as intratumoral injections of [redacted] in 0.5 mL per treatment. Following Cycle 1, if no accessible lesion for INXN-2001 injection is present (eg, due to complete resolution), then INXN-2001 should be injected into a tumor-involved

draining lymph node of a previously accessible lesion. The injections will be administered on the first day of each cycle throughout the study.

Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted] Blood pressure should be monitored Cycle 1 and 2 Days 1, 2, 3 or 4, and regularly thereafter.

8.2.4 Method for Assigning Subjects to Dose Level Cohorts

Subjects will be centrally assigned to dose level cohorts in accordance with the dose escalation scheme for this study.

8.2.5 Preparation and Administration of Study Drug

A combination of two investigational medications will be evaluated for safety, tolerability, efficacy, and biological effects in this trial. The small molecule INXN-1001 will be administered as oral capsules to subjects in combination with an intratumoral injection of INXN-2001 (on Day 1 of each cycle). For each treatment cycle, INXN-2001 dosing will occur 3 hours ± 30 minutes after the first dose of INXN-1001.

8.2.5.1 Preparation and Administration of INXN-1001

INXN-1001 will be provided by the sponsor in gelatin capsules of three different strengths (5 mg, 10 mg and 20 mg).

INXN-1001 capsules will be dispensed for subject oral dosing by the site pharmacy. The site must instruct the subject to take each dose in a fed state (30 minutes after the start of a normal meal) and to not eat any food for at least 4 hours after each dose is taken.

8.2.5.2 Monitoring of Subject Adherence and Managing Missed INXN-1001 Doses

The first daily dose of INXN-1001 at each treatment cycle is expected to be administered to the subject at the clinical site, under careful medical supervision by the clinic staff to ensure that the subject does not have difficulty with the size or quantity of capsules to be administered. Thereafter, subjects may be allowed to self-administer the remaining 6 doses at approximately the same time and in the same relationship to meals as administered at the clinical site on Day 1 of the treatment cycle. Subjects are to be instructed to take all of the capsules in the same way for the remaining treatment period.

Subjects should be instructed to make up for a missed dose by taking the dose the following day, and then continuing with dosing as scheduled (eg, if the dose on Day 3 is missed, dosing should occur on Day 4 and then continue as scheduled on Days 5, 7, 9 etc.).

Subjects will also be instructed to bring the treatment container(s) back with all remaining capsules at the next visit, to provide an assessment of the degree of adherence. All subjects will be queried about dose schedule adherence and reasons for missed doses.

8.2.5.3 Preparation and Administration of INXN-2001

INXN-2001 will be supplied by the sponsor as single dose vials. Information regarding the preparation of the INXN-2001 dose will be provided in the Pharmacy Manual.

INXN-2001 [redacted] will be administered as an intratumoral injection 3 hours (± 30 minutes) after oral INXN-1001 dosing on Day 1 of each cycle. INXN-2001 should be injected into a different lesion at each cycle.

If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. If only 2 accessible lesions are present at screening and the single injected lesion can no longer support the injection volume at subsequent cycles, then the medical monitor should be contacted for a discussion.

Note: One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001.

The INXN-2001 intratumoral administration should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or tumor-involved lymph node, at approximately 0.5 cm apart three dimensionally, to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. Radiographically-guided injections are permitted under BL2 conditions. A detailed description and photograph of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions should be documented. Lesions displaying signs of local infection should not be injected.

Should the tumor selected for injection not support the entire INXN-2001 injection volume, another tumor should be injected with the remaining volume to ensure that all subjects receive [redacted]. If another tumor is not available, then the remaining volume should be injected into a draining lymph node of the injected tumor. Following Cycle 1, if no accessible lesion for INXN-2001 injection is present (eg, due to complete resolution), then INXN-2001 should be injected into a draining lymph node of a previously accessible lesion.

Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions, for at least 2 hours following the INXN-2001 injection. The subject should be instructed to call the clinical site if any such reactions develop or don't resolve within 24 to 48 hours.

8.2.6 Retreatment Criteria, Dose Delays and Modifications

Subjects may receive up to 6 cycles of study drug treatment. In order to receive each repeat treatment cycle, all treatment-related adverse events must have resolved to Grade 1 or baseline and the following laboratory criteria must be met:

- Hemoglobin ≥ 9 g/L
- Lymphocytes > 700/ mm^3
- Neutrophils ≥ 1,500/ mm^3
- Platelets ≥ 100,000/ mm^3
- Serum creatinine ≤ 1.5 x ULN
- AST and ALT ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5×ULN
- Total bilirubin < 1.5 x ULN
- INR and PTT <1.5 x ULN, if not therapeutically anticoagulated.

Clinical laboratory tests drawn for analysis on Day 15 of each cycle may be used for determining if the retreatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration. Treatment delays to allow for recovery to acceptable levels are allowed at weekly intervals up to a maximum of 4 weeks. In the event that a treatment-related dose delay is necessary, then the next cycle of study treatment will be administered at the next lower INXN-1001 dose level. Subjects in the first dose cohort who do not meet the retreatment criteria will be discontinued from study treatment (ie, no dose reductions are permitted).

Subjects who experience a DLT will be managed as described in Section 6.2.4. Subjects who experience an injection site reaction should be managed as described in Section 8.2.7.

Subjects who experience a treatment-related Grade 3 adverse event, other than nausea and/or vomiting in the absence of optimal treatment with anti-emetics, will be discontinued from study treatment. If, however, the subject has experienced a substantial benefit from treatment and the SRC recommends continuation on the study, re-exposure to the experimental treatment may proceed on a case by case basis.

Subjects who experienced a Grade 4 treatment-related adverse event at any time during study treatment must be discontinued from study treatment.

8.2.7 Severity Grading and Management of Injection Site Reactions

Injection of a biologic agent carries a potential risk of injection site reaction which is characterized as an intense reaction (usually immunologic) at or near the site of injection. Injection reactions will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Table 3 outlines the injection site reaction grading system, associated symptoms, and the recommended actions to be taken. For any injection reaction, a serum tryptase level will be measured. Reactions at the INXN-2001 injection site that occur within 24 hours after the injection and are attributed to INXN-2001 will be considered to be injection related AEs.

Table 3. Injection Site Reaction Severity Grading and Management

CTCAE Grade	Symptoms	Course of Action
1	Tenderness with or without associated symptoms (eg, warmth, erythema, itching)	No intervention required.
2	Pain; lipodystrophy; edema; phlebitis	Proceed with further dosing cycles.
3	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Discontinue further study treatment and notify Medical Monitor. Report and manage as DLT: see Section 6.2.4. If toxicity meets seriousness criteria, immediately report as per SAE reporting procedures; see Section 9.4.
4	Life-threatening consequences; urgent intervention indicated	Permanently discontinue study treatment and notify Medical Monitor. Report and manage as DLT: see Section 6.2.4. Defaults to "serious". Immediately report as per SAE reporting procedures; see Section 9.4.
5	Death	Immediately notify Medical Monitor and report as per SAE reporting procedures; see Section 9.4. Discontinue further subject enrollment as per study stopping rules; see Section 6.2.7.

As with all AEs, injection-related signs or symptoms which, in the Investigator's judgment, are not related to immunologic reactions (eg, dyspepsia, urinary frequency, etc) should be recorded and graded as AEs according to NCI CTCAE v.4.03 criteria in the CRF. Study stopping rules will not apply to a specific event if it is clearly unrelated to INXN-2001 injection (eg, accidental trauma). If an AE associated with injection cannot be definitely shown to be unrelated to INXN-2001, then study stopping rules will apply.

8.2.8 Prophylactic Hydration, Antipyretic and/or Analgesic Administration

The use of antipyretics and/or analgesics and aggressive hydration is allowed anytime during study treatment, as indicated, including prophylactic administration.

Since fever and flu like symptoms are commonly experienced following adenoviral vector administration it is strongly recommended that subjects be treated with prophylactic oral hydration, antipyretic and/or analgesic medications prior to study drug administration.

For those subjects who experience treatment related symptoms (eg, fever, headache, chills, dehydration, etc) during Cycle 1, prophylactic hydration, antipyretic and/or analgesic medications must be given prior to study drug administration in subsequent cycles. Please refer to Appendix 3 for a recommended regimen for the prophylactic hydration and administration of antipyretics and/or analgesics.

8.2.9 Disposition of Unused Study Drug

All unused study drug should be destroyed on site in accordance with applicable site facility and US Occupational Safety and Health Administration (OSHA) procedures after a full accountability has been documented. Any on-site destruction of unused study drug must be documented and the records maintained in the Investigator's study file.

8.3 CONCOMITANT THERAPY

Concomitant medication information, including blood products, vitamins and other supplements, will be collected for the time period beginning 28 days prior to the first dose of study drug through the Post-Treatment Safety Assessment visit. The following medications are or are not permitted:

Permitted:

- Subjects may receive standard treatments, including palliative and supportive care for any underlying illness with the exception of palliative radiotherapy which is NOT permitted.
- Antidiarrheal therapy is permitted for study drug-induced diarrhea.
- Antiemetics are permitted for study drug-induced nausea and vomiting.
- Treatment with vitamin/mineral supplements is acceptable provided that they do not interfere with study endpoints, in the opinion of the investigator.

Not Permitted:

- Subjects may not receive medications, foods or drinks that induce, inhibit, or are substrates of the CYP450 3A4 pathway within 7 days prior to the first dose of study drug through 96 hours after their last dose of INXN-1001. See Appendix 2 for examples.
- Subjects may not receive any other investigational agent or anti-cancer therapy (chemotherapy, radiotherapy, etc) while receiving study treatment.

8.4 EVALUATION AND ASSESSMENT PROCEDURES

8.4.1 Schedule of Assessments

A tabular schedule of evaluations and procedures is provided in Table 2 (Schedule of Assessments).

8.4.2 Safety Evaluations

8.4.2.1 Demographics and Medical History

Each subject's complete medical history will be documented during screening, including demographic information, relevant medical history, current primary cancer diagnosis, and prior cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries). In addition concomitant medications, including blood products, vitamins and other supplements, received during the 28 days prior to initiating study treatment will be recorded.

8.4.2.2 Physical Examinations

A complete physical exam will be performed at Screening and at the Post-Treatment Safety Assessment visits. Symptom-directed physical exams will be performed at Days 1, 8 and 15 of each cycle.

8.4.2.3 ECOG Performance Status

ECOG performance status will be assessed as provided in Appendix 1.

8.4.2.4 Height and Weight

Height (cm) will be measured during screening. Weight (kg) will be measured at screening, Day 1 of each cycle, and at the Post-Treatment Safety Assessment visit.

8.4.2.5 Vital Signs

Vital signs will include blood pressure, pulse, temperature, and respiration. Blood pressure is to be monitored closely to prevent hypotension for up to 72 hours after administration of INXN-1001. Blood pressure assessment is required on Day 3 or Day 4 for Cycle 1 and Cycle 2. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted]

8.4.2.6 Adverse Events

Monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. AEs/SAEs that occur following informed consent until the Post-Treatment Safety Assessment must be recorded on the AE CRF; AEs/SAEs that occur prior to informed consent should be added to the medical history CRF.

Definitions, documentation, and reporting of AEs and SAEs are described in Section 9.0.

8.4.2.7 Pregnancy Testing

Females of childbearing potential will have a serum pregnancy test at the screening visit and a urine pregnancy test on the first day of each treatment cycle, prior to administration of INXN-1001.

8.4.2.8 Hematology

Hematology tests include: complete blood count and white blood cell count, differential white blood cell count, red blood cell count, hematocrit, hemoglobin, red blood cell indices, reticulocyte count, MCV (mean corpuscular volume) and platelet count. PTT (partial thromboplastin time) and INR (international normalized ratio) will also be evaluated.

8.4.2.9 Serum Chemistry

Serum chemistry tests include: aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, blood urea nitrogen, glucose, sodium, potassium, chloride, calcium, phosphorus and bicarbonate.

8.4.2.10 Urinalysis

Urinalysis panel (dipstick) includes: appearance, pH, specific gravity, glucose, protein/albumin, blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated.

8.4.2.11 Electrocardiogram (ECGs)

A standard, single 12-lead ECG for local safety assessment will be done at screening, 2½ to 3 hours after INXN-1001 dosing (prior to INXN-2001 injection) on Day 1 of each cycle, and at the Post-Treatment Safety Assessment visit. The ECGs will be used to evaluate the QT/QTc interval.

8.4.2.12 Serum Tryptase

A serum tryptase level will be obtained upon the occurrence of a suspected injection reaction and/or hypersensitivity reaction.

8.4.2.13 Immune Response Analyses

Blood samples will be collected from the subjects at specified visits to evaluate the potential antibody and cellular immune response to INXN-2001 (adenovirus and, RTS components) and melanoma-associated antigens. [redacted] Plasma and/or serum cytokine levels (eg, IL-12, IFN-γ, etc) will be assayed using multiplex and/or ELISA methodology. One or more serum cytokines may be assessed in as close to real time as possible for one or more time points in a dosing cycle at the request of the Medical Monitor or other sponsor representative(s).

In addition to these planned analyses, [redacted]

8.4.3 INXN-1001 Pharmacokinetic Assessment

Subjects will undergo an INXN-1001 pharmacokinetic (PK) assessment during Cycle 1 of their study treatment. Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day 15 as defined in the Schedule of Assessments (see Table 2).

8.4.4 Efficacy Assessments

Appropriate cancer staging procedures should be performed during screening. For the purpose of this clinical trial, the following imaging is expected at screening:

a. CT of the chest, and CT (or MRI) of the abdomen and pelvis
b. MRI (or CT) of the brain if brain metastasis are known or suspected
c. CT or MRI of other anatomical regions as clinically indicated

For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines.[1] Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable).

For each subject, disease sites are to be assessed throughout the study using the same method(s) of assessment used at screening. Specific image acquisition guidelines will be provided by a central imaging laboratory. Chest, abdomen, pelvis imaging is required for all follow-up imaging time points; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated.

All subjects will have tumor response assessments performed including Cycle 3 Day 13, the Post-Treatment Safety Assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment and again at the Follow-Up Tumor Assessment visit (35 ± 7 days following the Post-Treatment Safety Assessment visit). The Investigator will evaluate each subject for response to therapy according to RECIST 1.1 guidelines.[1] Subjects without objective evidence of disease progression at the Follow-Up Tumor Assessment visit should continue to have tumor assessments performed at 8-10 week intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first.

In addition to radiological imaging techniques, photographs of visible cutaneous lesions should also be obtained. Digital photograph(s) with included measuring tape should be taken of all visible injected tumor(s) and non-injected tumors, and of any remaining visible local reactions in or around the injected lesion(s). Any subject who has or develops vitiligo, should be documented the same way. Details regarding photographic methodology will be provided in the Photography Operations Manual.

As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, the sponsor will be analyzing anti-tumor response by assessing total tumor burden over time. Please refer to Appendix 4 for a description of "immune-related Response Criteria" (irRC).

8.4.5 Assessments of Transgene Function, Immunological Activities and Biological Effects

8.4.5.1 Transgene Function

Punch biopsies of the tumor(s) and/or associated tumor involved draining lymph nodes will be collected for *in vivo* assessment of the presence of the adenoviral vector, possible ongoing transgene expression of hIL-12, and expression of IL-12-induced down-stream genes. The presence of the adenoviral vector will be assessed either by quantitative PCR using primer-specific probes for the RTS[redacted] switch components, to distinguish between exogenous versus endogenous adenovirus, or by IHC. Expression of IL-12 and IL-12-induced gene expression will be evaluated via qRT-PCR, using a panel of known IL-12-regulated genes in melanoma as well as signaling pathways downstream from IL-12.

The tumor biopsies will be analyzed with appropriately designed primers, including genes known to be regulated by IL-12. If sufficient material is available, microarray analyses may be performed to evaluate global changes in gene expression profiles after treatment.

8.4.5.2 Immunological Activities

Part of the biopsied tumor(s) will be evaluated by standard light microscopy and immunohistochemistry to assess cellular infiltration in the tumor. Cellular infiltration by effector cells, such as T cells and their subsets (CD4+, CD8+ and CTLs as assessed by granzyme B expression) and NK cells, will be evaluated as well as immune suppressor elements, such as T-regulatory cells (Tregs). Biopsy specimens will also be evaluated for IL-12 expression and markers of immune activation. Biopsy sections will be read by a pathologist who is unaware of study subject background and subjected to automated enumeration of positively staining cells. Blood will be drawn for assessing cell-mediated immunity, especially frequency of CTLs and Tregs. Unseparated peripheral blood mononuclear cells will be tested by flow cytometry for the relative percentages of immune cells, and if possible for intracellular IFN-γ, other cytokines and granzyme B, as characteristics of CTL, both directly after isolation and after in vitro stimulation with autologous DCs transduced by adenoviral vectors expressing major melanoma specific antigens. Unseparated peripheral blood mononuclear cells will also be stimulated with autologous DCs transduced by adenoviral vectors expressing major melanoma specific antigens in IFN-γ ELISPOT and/or flow cytometric intracellular staining for IFN-γ and other relevant cytokines, to enumerate the anti-melanoma-CTLs.

[redacted]

9. ADVERSE EVENTS

9.1 ADVERSE EVENT (AE) DEFINITION

An adverse event is any untoward medical occurrence associated with the use of a drug in humans whether or not considered drug related. Any worsening of a preexisting condition, which is temporally associated with the use of the study drug, is also an AE.

Adverse events include:

- Suspected adverse drug reactions;
- Reactions from study drug overdose, abuse, withdrawal, sensitivity, or toxicity;
- Significant changes or abnormalities when compared to baseline, in signs, symptoms, clinical laboratory results, or physiological testing. This includes any worsening of a preexisting condition temporally associated with the use of study drug;
- Other untoward medical events, regardless of their relationship to the study drug, such as injury, events that require surgery, accidents, extensions of symptoms, or apparently unrelated illnesses.

The following considerations apply when identifying an AE:

- Anticipated day-to-day fluctuations of pre-existing conditions including the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered adverse events.
- In the event that a constellation of symptoms results in a confirmed diagnosis, the diagnosis (not the symptoms) should be recorded as the adverse event term.
- If a diagnosis cannot be established, the symptoms should be recorded as the adverse event(s).
- If an ongoing symptom has been included in the medical history, an associated severity grade and frequency should also be documented so that a worsening in severity or frequency of a symptom can be readily identified as an AE.
- Progression of disease is not itself an AE however, the presenting sign or symptom of the disease progression should be documented as an AE (eg, increase in pain). *Exception:* If a subject experiences progression of disease that results in death, 'progression of disease' may be reported as an SAE if, by medical opinion, the term best describes the cause of death.

9.2 EVALUATION OF ADVERSE EVENTS (AEs)

9.2.1 Determination of Seriousness

The Investigator will determine the seriousness of an AE based on the following:

9.2.1.1 Serious Adverse Event (SAE)

An AE is considered an SAE if at least one of the following conditions applies:

- Death: An AE that results in death during the active study period or within 30 days following Study Drug administration. In addition, a reported death at any time post-study that is thought to be related to Study Drug administration;
- Life-threatening adverse event: An AE that places the patient, in the view of the Investigator, at immediate risk of death from the event as it occurred (ie, this does not include a reaction that had it occurred in a more severe form, might have caused death);
- Permanent, persistent, or significant disability: A disability is defined as any substantial disruption of a person's ability to conduct normal life functions;
- Inpatient hospitalization or prolongation of existing hospitalization: In general, hospitalization refers to admission of a patient into a hospital for at least a 24-hour stay. Hospitalizations for routine blood transfusions, hospitalization for an elective or diagnostic procedure, or surgery for a pre-existing condition that has not worsened, are not considered SAEs. (Emergency Room visits that do not result with admission are not considered as SAEs);
- A congenital anomaly/birth defect: A fixed, permanent impairment established at or before birth;
- Important medical event: Events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they jeopardize the patient and require medical or surgical intervention to prevent a life-threatening situation, hospitalization or death;
- New Cancer: Occurrence or diagnosis of a new cancer during the trial is considered a serious adverse event. (This does not pertain to metastasis of current disease);
- Any AE associated with an overdose of the study drug: An overdose of study drug is defined as an occurrence of administered dose exceeding that which is prescribed by the Investigator per protocol.

9.2.1.2 Non-Serious Adverse Event

Adverse events that do not fulfill the previous criteria are classified as non-serious AEs.

9.2.2 Determination of Severity

The severity of AEs will be assessed according to the NCI CTCAE, v. 4.03. If the AE is not defined in the NCI CTCAE, v. 4.03, the Investigator will determine the severity of an AE based on the following definitions:

- Mild (Grade 1): The AE is noticeable to the patient, but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the study drug.
- Moderate (Grade 2): The AE interferes with routine activity, but responds to symptomatic therapy or rest. The AE may require reducing the dose, but not

discontinuing administration of the study drug.

- Severe (Grade 3): The AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy. In addition, the AE leads to discontinuing administration or reducing the dose of the study drug.
- Life Threatening (Grade 4): The AE requires discontinuing administration of the study drug. The patient is at immediate risk of death.
- Death (Grade 5): The patient dies as a direct result of the complication or condition

Adverse events should be reported using the maximum intensity of the event (eg, if a subject reported nausea lasting 3 days, one start date and stop date should be recorded along with the maximum intensity experienced for that event over that 3 day timeframe).

9.2.3 Causality Assessments

The Investigator will use medical consideration to determine the potential relationship of the AE to the study drugs based on his/her clinical judgment. Assessment of causality will be based upon the following:

- Alternative possible causes of the AE, including the subject's underlying disease or co-morbid conditions, other drugs, other host and environmental factors;
- The temporal sequence between the exposure to study drug and the AE;
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the study drug (or similar drugs);
- Whether the AE resolved or improved with decreasing the dose or stopping the study drug (ie, dechallenge); or recurred or worsened with re-exposure to the drug (ie, rechallenge).

The relationship between the study drug and the AE will be described using one of the following categories:

- Unrelated: Another factor is clearly the cause of the AE.
- Possibly Related: There is a reasonable possibility that the study drug is the cause of the AE, including that the study drug and another factor(s) are equally likely as causes of the AE.
- Related: The AE can be fully explained by the administration of study drug.

9.3 DOCUMENTING ADVERSE EVENTS (AES)

All AEs (including SAEs) are to be accurately recorded on the Adverse Event page of the patient's eCRF from the time the patient signs the informed consent until the post-treatment safety visit. Each event will be assessed for serious criteria, severity, and causality (See "Causality Assessments"). The date of onset, as well as the duration of the event will be recorded. In addition, treatments provided to the patient, actions taken with the study drugs, and the outcome of the AE will also be noted.

9.4 REPORTING SERIOUS ADVERSE EVENTS (SAEs)

Time Frame for Reporting

SAEs must be reported to the Sponsor or Sponsor's designee within 24 hours of becoming aware of the event (regardless of the initiation of any new anti-cancer therapy) including the following:

- Any death or SAE experienced by the patient from the signing of informed consent to 30 days after the last dose of study drug, regardless of relationship to study drug.
- Any death or SAE that the Investigator becomes aware of, and believes to be study drug related, that occurs more than 30 days after the patient last received study drug.

Study drug-related AEs/SAEs that are ongoing at the time of the Post-Treatment Safety Assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized or become chronic (and following consultation and agreement by the ZIOPHARM Medical Monitor).

Information to be provided by the Investigator

Within 24 hours of becoming aware of the SAE or patient death, the Investigator must notify the Sponsor or designee and transmit information to the Sponsor or designee. Information (initial and follow-up) should be provided on an electronic and/or paper SAE Report form signed and dated by the Investigator. The SAE Report form and copies of source documents (with patient identifiers redacted) will be transmitted electronically or by fax. A hospital discharge summary should be provided if the subject was hospitalized. An SAE report will be considered final once all relevant information has been received and reviewed by the Sponsor.

The SAE Report form is provided in the Investigator study files. Please refer to the Investigator study files for instructions on how to complete the form. The Investigator will provide all of the following information related to the event:

- Investigator identification
- Patient identification (eg, patient number, initials, sex, age or date of birth)
- Information regarding study drug administration (eg, start/stop date, dose and frequency)
- Description of event
- Action taken with the study drugs in relation to the SAE
- Outcome of the SAE

In addition to the above information, the Investigator must provide, for each event term, an assessment of:

- Severity/intensity
- Relationship to the study drug (causality assessment)

9.5 SPONSOR AND INVESTIGATOR RESPONSIBILITY FOR REPORTING ADVERSE EVENTS (AEs)

All AEs will be reported to regulatory authorities, IRBs/IECs, and Investigators in accordance with all applicable global laws and regulations. The Investigator must submit all Safety Letters from the Sponsor to his/her IRB/IEC per agreements and local requirements. The Investigator must keep copies of all safety reports, including correspondence with ZIOPHARM and the IRB/IEC, in the study file.

9.6 FOLLOW-UP INFORMATION

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has resolved, stabilized, returned to baseline, or is otherwise explained by the Investigator.

Follow-up data concerning the SAE (ie, diagnostic test reports, hospital summaries, etc) must be promptly reported (within 24 hours of receipt) to the Sponsor or Sponsor's designee, until resolution of the SAE. Should the FDA or National Regulatory Authorities require that the Sponsor submit additional data on the event, the Investigator will be asked to provide those data to the Sponsor in a timely fashion.

Required Follow-up for Serious Adverse Events (SAEs)

All treatment-related AEs/SAEs that are ongoing at the time of the Post-Treatment Safety visit will be followed until:

- The event resolves, or
- The event returns to baseline if a baseline value is available, or
- The event stabilizes (and following consultation and agreement by the ZIOPHARM Medical Monitor), or
- The event can be attributed to factors other than the study drug or other than study procedure.

9.7 PREGNANCIES

Subjects who become pregnant during the study should immediately discontinue participation in the study. The Sponsor should be immediately notified.

An initial Pregnancy Report form and a Pregnancy Outcome Form are to be completed by the Investigator or designee. The Pregnancy Report form and the completion guidelines will be provided in the investigator study files. Please refer to the investigator study files for details on how to complete these forms.

10. STATISTICAL PROCEDURES

Data from this study will be analyzed and included in a clinical study report that will be prepared after the last subject has completed the Post-Treatment Safety Assessment visit. Additional data collected from subjects who have not progressed by the Post-Treatment Safety Assessment visit and are continuing to be followed for tumor response at the time of the final data cut-off date will be presented in an addendum to the study report.

10.1 DETERMINATION OF SAMPLE SIZE

No formal sample-size estimation was performed. The choice of the number of subjects was based on the standard Phase I 3+3 inter-cohort dose escalation design in which 3 to 6 subjects are enrolled into each dose level cohort.

In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. Based on a review of available safety and clinical response data, an additional 15 subjects may be enrolled in Phase II at a single dose level at or below the MTD. Should a subject discontinue study treatment before receiving 3 cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis.

Given these considerations, a total of approximately 30 subjects may be enrolled into this study, including 6 subjects enrolled in each of the 4 planned dose level cohorts and approximately 15 additional subjects enrolled at a single dose level at or below the MTD. Additional subjects may also be enrolled as replacement subjects or to explore intermediate dose level cohorts, if any.

10.1.1 Subject Randomization

Not applicable.

10.1.2 Populations for Analysis

The following subject populations will be evaluated and used for presentation and analysis of the data:

- Safety Population: All subjects who receive at least one INXN-1001 capsule or, in the event an injection of INXN-2001 is administered before an INXN-1001 capsule is taken, at least one injection of INXN-2001.
- Activity Evaluable Population: All subjects who received at least 1 dose of INXN-2001 and INXN-1001, and have at least 1 post-screening response evaluation.

10.2 PROCEDURES FOR HANDLING MISSING, UNUSED, AND SPURIOUS DATA

No imputation of values for missing data will be performed. Standard clinical monitoring and data management practices will be used to ensure the integrity of data.

10.3 STATISTICAL METHODS

Categorical data will be summarized using counts and percentages based on non-missing values. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented.

Data will be summarized by dose cohort based on the actual dose of INXN-1001 received on Day 1, Cycle 1. For Phase II, data will be summarized based on group and dose regimen.

10.3.1 Baseline Characteristics

Demographic and baseline disease characteristic data will be summarized. Data to be tabulated will include at least demographic features such as sex, age, and race, as well as disease-specific status and medical history.

10.3.2 Safety Analyses

The Safety Population will be used for the analyses of safety data based on the dose of INXN-1001 received.

Safety evaluations will be based on the incidence, intensity, and type of AEs and SAEs. Clinically significant changes in the subjects' physical examinations, vital signs, and ECG evaluations, and clinical manifestations relevant to abnormal laboratory values will be captured as AEs. Safety will also be assessed based on medical history and prior/concomitant medications.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) for the purpose of summarization. All AEs occurring during the study will be included in by-subject data listings. Treatment-emergent events will be tabulated. 'Treatment-emergent' is defined as any AE that occurs during or after administration of the first dose of study drug through the Post-Treatment Safety Assessment visit (28 days after the last INXN-1001 dose); any event after the first dose of study drug that is considered study drug-related regardless of the start date of the event; or any event that is present at baseline that worsens in intensity or is subsequently considered to be drug related by the investigator. Deaths, SAEs, and AEs resulting in study discontinuation will be listed.

Safety variables will be tabulated and presented by dose cohort. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated.

10.3.3 Efficacy Analyses

The Activity Evaluable Population will be used for the analyses of efficacy data based on the dose of INXN-1001 received.

Following completion of the study, best response will be determined for each subject in accordance with RECIST v.1.1 guidelines and the objective response rate presented for each dose cohort. Progression-free survival and durability of response will be determined using Kaplan-Meier methodology.

As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, the sponsor will be analyzing anti-tumor response by assessing total tumor burden over time. Please refer to Appendix 4 for a description of "immune-related Response Criteria" (irRC).

10.3.4 Immunologic Responses

The trial will assess the immunological effects of each treatment cycle on the cellular and humoral immune responses in the peripheral circulation and in tumor biopsy specimens. Humoral immune responses (potential antibody production) to the adenovirus and/or the RTS[redacted] components as well as against melanoma antigens will be compared to baseline in serum samples, and cytokine profiling will also be performed by multiplex analysis. Whole blood will be collected for flow and/or ELISPOT assays to test anti-tumor T lymphocyte responses against adenovirus and/or the RTS[redacted] components as well as against melanoma-associated antigens. Samples will also be screened for the relative percentages of mononuclear immune cell subpopulations. At each time point, the change in immunologic response (CTL and Treg frequency in blood) from baseline and from the preceding time point will be correlated with the INXN-1001 dosage level.

In addition, tumor punch biopsies or FNAs will be examined for genomic changes due to IL-12 expression, presence of injected adenovirus and CTL frequency, Treg and MDSC frequency, and other immunological markers. The change in each measure from baseline and from the preceding biopsy will be correlated with the INXN-1001 dose used in the each cohort.

10.3.5 INXN-1001 Pharmacokinetics

INXN-1001 PK parameters to be determined will include, but is not limited to, the maximum concentration (C_{max}), time to maximum concentration (T_{max}), half-life ($t_{1/2}$), area-under-the-concentration time curve (AUC), volume of distribution (V_d), and clearance (CL). Where possible, descriptive statistics of the PK parameters will be provided; individual subject INXN-1001concentrations, actual sampling times, and PK parameters will be listed.

10.3.6 Multi-Center Study

Efficacy and safety data will be presented for each dose cohort pooled over all centers.

10.3.7 Adjustments for Covariates

No adjustments for covariates will be made.

10.4 PROCEDURES FOR REPORTING DEVIATIONS TO ORIGINAL STATISTICAL ANALYSIS PLAN

A formal statistical plan for the analysis and presentation of data from this study will be prepared prior to database lock. Deviations from the statistical analyses outlined in this protocol will be indicated in this plan; any further modifications will be noted in the final clinical study report.

11. STUDY ADMINISTRATION

11.1 CASE REPORT FORMS AND SOURCE DOCUMENTATION

For each subject, case report forms and corresponding source records will be kept and will include:

- Drug treatment (dose and dates)
- Concomitant drug therapies
- Data from the Post-Treatment Safety Assessment
- Toxicity records (clinical and autopsy dates, when available)
- Other pertinent data

Case report forms should be completed in a timely manner, and every effort should be made to have forms completed and up-to-date in anticipation of a visit by the sponsor's monitor. Specific instructions will be provided to the site.

All requested information must be entered on the CRF in the spaces provided. If an item is not available or is not applicable, it should be documented as such; do not leave a space blank. The completed CRF must be promptly reviewed, signed, and dated by the investigator. It is the obligation of the investigator to review each page of the CRF. Case report completion may be delegated to other study personnel; however, the sponsor must be informed in writing of the name of such persons and the scope of their authority. If, for any reason, certain data are lacking to complete an individual report form, the investigator will provide a written statement explaining the reasons for the lack of data.

11.2 GOOD CLINICAL PRACTICE STATEMENT

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, applicable regulatory requirements, and ZIOPHARM policies.

11.3 SPONSOR MONITORING

After satisfactory receipt of all necessary regulatory paperwork, the sponsor's monitor will arrange that all study material be delivered to the study site at a mutually convenient time. An initiation visit by ZIOPHARM and its monitoring personnel will be made. At this meeting, all personnel expected to be involved in the conduct of the study will undergo an orientation to include review of study protocol, instruction for CRF completion and overall responsibilities, including those for drug accountability and study file maintenance.

Throughout the course of the study, the sponsor's monitor will make frequent contact with the investigator. This will include telephone and/or on-site visits. During these visits, CRFs will be reviewed for completeness and adherence to protocol. As part of the data audit, it is expected that source documents (eg, hospital records, office records) will be made available for review by the monitor. The monitor also will perform drug accountability checks, and may periodically request review of the investigator's study file to assure completeness of documentation in all respects of study conduct.

Upon study completion, the monitor will arrange for a final review of the study files, after which the file should be secured by storage for the appropriate period as specified in Section 12.6. The investigator or appointed delegate will receive the sponsor's representative during these on-site visits and will cooperate in providing the documents for inspection and responding to inquiries that may arise as part of this review. The investigator will also permit inspection of the study files by authorized representatives of the FDA.

11.4 DURATION OF THE STUDY

The study is estimated to complete enrollment over 24 months. The estimated number of months to complete study visits for the last enrolled subject is approximately 9 months (assuming 6 treatment cycles). Therefore, the total duration of this study is expected to be approximately 1.5 to 2.5 years.

Each subject's participation in this study will last approximately 8 months, including:

- 30 day screening period.
- 6 cycles (24 weeks) of study treatment.
- Post-Treatment Safety Assessment visit performed 28 days after the last dose of INXN-1001.
- Follow-Up Tumor Assessment visit performed 35 ± 7 days after the Post-Treatment Safety Assessment visit.

In addition, subjects who discontinue or complete study treatment without objective evidence of disease progression should continue to be followed until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first. The active study period refers to the study period from informed consent through the Post-Treatment Safety Assessment visit.

11.5 RECORDS RETENTION

Records of drug disposition, CRFs, and reports of the clinical trial must be maintained by the investigator for a period of at least two years following the date on which the test article is approved by FDA for marketing for the purposes that were investigated in the study. If no application is to be filed or if the application is not approved for such indication, the records must be stored for two additional years and then returned to ZIOPHARM. No records will be destroyed, but will be indefinitely stored.

11.6 INSTITUTIONAL REVIEW BOARD

This protocol and the study informed consent form must be reviewed and approved by the Institutional Biosafety Committee (where applicable) and IRB/IEC prior to the start of the study and a copy of the approval letter supplied to ZIOPHARM. During the course of the study, the investigator shall make timely and accurate reports to the IRB/IEC on study progress at intervals not exceeding one year, as well as satisfying any other local IRB reporting regulations. Copies of all reports to, and correspondence with, the IRB/IEC must be provided to ZIOPHARM. Further, within three months of the completion or early termination of the study, a final report should be made to the IRB/IEC and ZIOPHARM by the investigator.

All protocol revisions must originate with and be documented by ZIOPHARM. If the requested revision is an amendment, the investigator must sign it. The FDA will be notified of all revisions by ZIOPHARM. The investigator must submit the amendment to his/her IRB/IEC for review and approval prior to implementation. Documentation of approval signed by the chairperson or designee of the IRB/IEC must be sent to ZIOPHARM.

It is the investigator's obligation to maintain an IRB/IEC correspondence file and to make this available for review to ZIOPHARM representatives as part of the routine study monitoring process.

11.7 CONFIDENTIALITY AND HIPAA

The written Informed Consent will explain that study data will be stored in a database, maintaining confidentiality in accordance with national data legislation. All data processed by ZIOPHARM, or its representative, will be identified by subject number and study code.

The written Informed Consent will also explain that for data verification purposes, authorized representatives of ZIOPHARM, a regulatory authority (FDA), and/or the IRB may require direct access to parts of the hospital or clinic records relevant to the study that include the subject's medical history.

The Informed Consent Form will be accompanied by or include a separate document incorporating US Health Insurance Portability and Accountability Act (HIPAA)-compliant wording by which the subjects authorize the use and disclosure of their Protected Health Information.

12. INFORMED CONSENT

12.1 FDA INFORMED CONSENT REQUIREMENTS

The investigator or his/her staff will explain the nature of the study, its purpose and associated procedures, the expected duration and the potential risks involved to the prospective subject prior to enrollment. It should also indicate that, by signature, the subject or, where appropriate, a legal guardian, permits access to relevant medical records by the sponsor and by representatives of the US FDA. If a prospective subject does not understand English, an appropriate translation into his or her primary language must be made available. The investigator or designee will obtain written, informed, and witnessed consent. The individual will have ample time and opportunity to ask questions. He or she will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Following the discussion regarding the study, the prospective subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the individual voluntarily agrees to sign the informed consent statement and has done so, may he/she enroll into the study. A copy of his/her signed and dated informed consent will be provided to each subject. The signed informed consent form is to remain in the investigator's file.

The informed consent form and any other written information provided to the subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or if there is an amendment to the protocol that necessitates a change to the content of the subject's informed consent. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm continuation of his/her participation in the study by his/her signature on the revised informed consent form (if applicable). Any written informed consent form and written information must receive IRB approval/favorable opinion in advance of use.

12.2 SUBJECT INFORMED CONSENT FORM

ZIOPHARM will provide a sample subject Informed Consent Form, for modification as appropriate by the investigator.

13. PROTOCOL APPROVAL PAGE

A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma

With the exception of a change intended to eliminate an immediate hazard to subjects, the study shall be conducted as described in the approved protocol. All deviations from the protocol will be documented in the CRF. Any significant deviation or deviation related to dosing or safety evaluation will be reported to ZIOPHARM and documented in the CRF.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to principles of Good Clinical Practice and local regulations and requirements.

Center Name: _____

Principal Investigator

Print Name: _____

Signature: _____

Date: _____

14. REFERENCES

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15. APPENDIX 1: EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS

ECOG PERFORMANCE STATUS[a]

Grade	Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead.

[a] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-55, 1982.

16. APPENDIX 2: CYP450 3A4 INDUCERS, INHIBITORS AND SUBSTRATES

Medications, foods and drinks which induce, inhibit or are substrates of the CYP450 3A4 pathway include, but are not limited to, the following:

Alfentanil
Alfuzosin (Uroxatral)
Almotriptan (Axert)
Alprazolam
Aminoglutethimide
Amlodipine (Norvasc)
Amprenavir
Aprepitant (Emend)
Astemizole
Atazanavir (Reyataz)
Atorvastatin (Lipitor)
Bepridil (Vascor)
Bexarotene (Targretin)
Bosentan (Tracleer)
Bromocriptine (Parlodel)
Budesonide (Entocort)
Buprenorphine (Subutex)
Bupropion /(Buspar)
Carbamazepine (eg, Tegretol)
Cerivastatin
Cevimeline (Evoxac)
Chloramphenicol
Cilostazol (Pletal)
Cisapride
Clarithromycin
Clonazepam (Klonopin)
Clopidogrel (Plavix)
Colchicine
Conivaptan
Danazol
Dapsone (Avlosulfon)
Darunavir (Prezista)
Dasatinib
Dasatinib (Sprycel)
Delavirdine
Dihydroergotamine
Diltiazem
Disopyramide (Norpace)
Donepezil (Aricept)
Droperidol
Dutasteride (Avodart)
Ebastine (Kestine)
Efavirenz (Sustiva)
Eletriptan (Relpax)
Eplerenone (Inspra)
Erythromycin
Estazolam (ProSom)
Eszopiclone (Lunesta)
Ethosuximide (Zarontin)
Felodipine
Fentanyl (Sublimaze)
Finasteride (Proscar)
Fluconazole
Fluoxetine
Flurazepam (Dalmane)
Fluvoxamine
Fosamprenavir
Fosamprenavir (Lexiva)
Fosphenytoin
Galantamine (Reminyl)
Granisetron (Kytril)
Grapefruit juice
Griseofulvin
Halofantrine (Halfan)
Ifosfamide (Ifex)
Indinavir
Isoniazid
Isradipine (DynaCirc)
Itraconazole
Ixabepilone (Ixempra)
Ketoconazole (Nizoral)
Lapatinib (Tykerb)
Levomethadyl (Orlaam)
Loperamide (Imodium)
Lopinavir (Kaletra)
Loratadine
Lovastatin
Maraviroc (Selzentry)
Mefloquine (Lariam)
Miconazole
Midazolam
Mifepristone (Mifeprex)
Modafinil (Provigil)
Nafcillin
Nefazodone
Nelfinavir
Nevirapine
Nevirapine (Viramune)
Nicardipine (Cardene)
Nifedipine (Adalat)
Nimodipine (Nimotop)
Nisoldipine (Sular)
Nitrendipine (Baypress)
Oxcarbazepine
Oxybutynin (Ditropan)
Oxycodone (Percodan)
Paricalcitol (Zemplar)
Phenobarbital
Phenytoin
Pimozide (Orap)
Pioglitazone
Posaconazole
Praziquantel (Biltricide)
Primidone
Propoxyphene
Quazepam (Doral)
Quetiapine (Seroquel)
Quinacrine
Quinidine
Quinine
Quinupristin
Ranolazine (Ranexa)
Repaglinide (Prandin)
Rifabutin (Rimactane)
Rifampin
Rifapentine
Ritonavir
Saquinavir
Sibutramine (Meridia)
Simvastatin
Sirolimus (Rapamune)
Sildenafil
Solifenacin (Vesicare)
St. John's wort
Sufentanil (Sufenta)
Tacrolimus
Tadalafil (Cialis)
Tamsulosin (Flomax)
Telithromycin
Teniposide (Vumon)
Terfenadine
Testosterone
Tiagabine (Gabitril)
Tinidazole (Tindamax)
Tipranavir (Aptivus)
Topiramate (Topamax)
Triazolam
Troleandomycin
Vardenafil (Levitra)
Verapamil
Voriconazole
Zafirlukast
Ziprasidone (Geodon)
Zolpidem (Ambien)
Zonisamide (Zonegran)
Zopiclone (Imovane)

17. APPENDIX 3: SUGGESTED REGIMEN FOR HYDRATION, ANTIPYRETIC AND/OR ANALGESIC PROPHYLAXIS

Recombinant adenoviral vectors have the potential to elicit potent cellular and humoral immune responses in recipients. While the mechanism responsible for these effects is poorly understood, dehydration and transient low-grade fevers are common after systemic recombinant adenovirus vector administration and temperatures up to 104^{o} F with chills and generalized malaise have been observed in first and/or subsequent treatment cycles.

Dehydration may occur in the absence of adequate hydration prophylaxis. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. Oral hydration is of paramount importance after first injection of INXN-2001 through Cycle 2 because most toxicity has been observed during this time period.

Because low grade fever is very likely to occur, prophylaxis with a non-steroidal anti-inflammatory agent (ibuprofen) or acetaminophen (if a subject cannot tolerate ibuprofen) is strongly recommended starting with Cycle 1.

- Ibuprofen is available without a prescription in 200 mg tablets. Usually 800 mg every 6-8 hours will prevent and/or decrease fever. The lowest expected efficacious dose should be used.
    - Side effects of ibuprofen include nausea and vomiting, which may be prevented if the medication is taken with food. Rare side effects include diarrhea, constipation, heartburn, and stomach pain. People with stomach ulcers or kidney disease, and those with an aspirin allergy should avoid ibuprofen.
    - Common brand names of ibuprofen include Advil®, Motrin®, and Nuprin®.
    - Aspirin should be avoided as it may be toxic in large doses in adults.
- While meta-analyses suggest that ibuprofen is a better anti-pyretic medication than acetaminophen, acetaminophen also prevents and or reduces a fever. It is available without a prescription in 325 mg or 500 mg tablets. Again, 1000 mg every 6-8 hours should be used to eliminate fever. The maximum dose of acetaminophen in adults should not exceed 4 grams in a 24 hour period.
    - Side effects are rare, but some people are allergic to the medication. Over doses may cause liver failure. Therefore, people with liver disease and chronic alcohol users should avoid this medication.
    - Common brand names of acetaminophen are Aspirin Free Anacin®, Feverall®, Genapap®, Panadol®, Tempra®, and Tylenol®.

In general, a fever can be treated with the same non-steroidal anti-inflammatory agents (eg, ibuprofen) or with acetaminophen. Alternating doses of ibuprofen with acetaminophen will also effectively control fever and prevent accidental overdose. If a fever occurs in spite of prophylactic medication or does not respond to usual doses of ibuprofen or acetaminophen then a combination of both acetaminophen and ibuprofen may be needed to stop the fever. If a fever does occur after administration of the adenoviral vector in any cycle then anti-pyretic prophylaxis should be used in all subsequent dose cycles.

18. APPENDIX 4: IMMUNE-RELATED RESPONSE CRITERIA (irRC)

Anti-tumor response based on total measurable tumor burden will be explored as a supplement to the standard RECIST v1.1 criteria established to evaluate anti-tumor responses to chemotherapeutic agents. Wolchok, et al maintain that by measuring total tumor burden over time this immune related response criteria (irRC) may prove to be a more sensitive means of evaluating antitumor response to immunotherapeutic agents. For the irRC, both index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden). The following appendix describes the process of deriving the irRC overall response as defined by Wolchok et al.[39]

At Baseline:

- Identify a maximum of 15 index lesions (up to 5 lesions per organ, up to 10 visceral lesions and 5 cutaneous lesions).
  - Index lesions must be measurable; where measurable is defined as ≥5 x 5mm.
- Calculate the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions.
- All remaining lesions are followed as non-index lesions.

At Each Subsequent Assessment: (see Table 2, Schedule of Assessments)

- Calculate the SPD of all index lesions.
- Identify a maximum of 10 new, measurable lesions.
  - Measurable is defined as ≥5 x 5mm.
  - Up to 5 new lesions per organ.
  - Up to 10 new visceral lesions and 5 new cutaneous lesions.
- Calculate the SPD of all new, measurable lesions.
- Calculate the total tumor burden.
  - **Tumor Burden = $SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$**

At each tumor assessment after baseline the response in index and new, measurable lesions will be defined based on the change in tumor burden. Decreases in tumor burden will be assessed relative to baseline measurements (ie, the SPD of all index lesions at screening).

The irRC overall response is derived from time point response assessments (based on tumor burden) as follows:

- irCR – complete disappearance of all lesions (whether measurable or not, and no new lesions) with confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.

- irPR – decrease in tumor burden ≥50% relative to baseline with confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.

- irSD – not meeting criteria for irCR or irPR, in absence of irPD.

- irPD – increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) with confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.

Derivation of irRC Overall Response:

Measurable Response	Non-Measurable Response		Overall Response
Tumor Burden[a]	**Non-Index Lesions**	**New, Non-Measurable Lesions**	**Using irRC**
↓100%	Absent	Absent	irCR[b]
↓100%	Stable	Any	irPR[b]
↓100%	Unequivocal Progression	Any	irPR[b]
↓≥50%	Absent/Stable	Any	irPR[b]
↓≥50%	Unequivocal Progression	Any	irPR[b]
↓<50% to <25%↑	Absent/Stable	Any	irSD
↓<50% to <25%↑	Unequivocal Progression	Any	irSD
≥25%↑	Any	Any	irPD[b]

[a] Decreases assessed relative to baseline and increases assessed relative to nadir, including measurable lesions only (≥5 × 5 mm).

[b] Assuming response (irCR and irPR) and progression (irPD) are confirmed by a 2nd consecutive assessment at least 4 wks apart.

In the absence of rapid clinical deterioration, irPD at the Post Treatment Safety Assessment visit should be confirmed by repeat scans at the Follow-Up Tumor Assessment to ensure that more slowly declining tumor burden in response to therapy is not missed.

19. APPENDIX 5: PHASE I AND PHASE II GROUP 1

Study Design for Phase I and Phase II Group 1

Single-arm, open label, Phase I/II dose escalation study of intratumoral injections INXN-2001 and oral INXN-1001 in subjects with unresectable Stage III or IV melanoma.

Table 1: Study Design

Study Part	Cohorts	Dose Regimen
Phase I	Dose Escalation	INXN-2001: [redacted] INXN-1001: 5, 20, 100, 160 mg
Phase II	Group 1	INXN-2001: [redacted] INXN-1001: 160 mg daily for 7 consecutive days; 21-day cycle
	Group 2	INXN-2001: [redacted] INXN-1001: 160 mg every other day for 14 days; 28-day cycle

In Phase I, four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to 6 treatment cycles, each of 21 days in duration. Previously enrolled subjects received 3 cycles under former versions of this protocol. There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. In each cycle, the subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001. No intrasubject dose escalation will be allowed.

In Phase II, approximately 15 additional subjects will be enrolled at a single dose level at or below the MTD. Phase II of the study will include two groups of subjects. Subjects enrolled in the Phase II portion of the study under Amendment 6 or prior will be enrolled into Group 1; subjects enrolled under Amendment 7 or later will be enrolled into Group 2.

Subjects in Group 1 will be treated with one intratumoral injection of INXN-2001 in combination with oral doses of INXN-1001 given at 160 mg daily for 7 consecutive days at the beginning of a 21-day cycle.

Dose and Schedule During Phase I and Phase II Group 1

Phase I: All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 3 week cycle. The subjects will also receive a single daily oral dose of INXN-1001 for 7 consecutive days starting on Day 1 of each cycle. Subjects may receive up to 6 cycles of study treatment.

Phase II Group 1: All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 3 week cycle. The subjects will also receive a single oral dose of INXN-1001 for 7 consecutive days starting on Day 1 of each cycle. Subjects may receive up to 6 cycles of study treatment.

Dose Escalation Plan During Phase I	Phase I: Four sequential INXN-1001 dose escalation cohorts are planned, as described in Table 1. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. No intrasubject dose escalation will be allowed. An intermediate dose level(s) of INXN-1001 may be explored after review of all available safety and activity data, as may be decided by the SRC. Initially, 3 subjects will be enrolled into each sequential dose cohort, beginning with the lowest planned INXN-1001 dose level. Dose escalation will not occur until the third subject in a cohort has completed the first treatment cycle (7 days of oral activator ligand followed by 14 days of observation). Following this, a meeting of the SRC will be convened to review all reported ≥Grade 3 adverse events to determine if these meet dose-limiting toxicity (DLT) criteria. In addition, the SRC will review the overall occurrence of adverse events and relevant laboratory data in each cohort to determine whether the MTD has been reached. Following this review, the SRC will advise on subject enrollment, dose escalation and/or cohort expansion using the following guidelines: a) If 0 of 3 subjects in a cohort experiences a DLT, then the next higher dose-level cohort may be enrolled. b) If 1 of 3 subjects in a cohort experiences a DLT, then up to 3 additional subjects will be enrolled into that cohort. c) If >1 of 3-6 subjects in a cohort experience a DLT, then enrollment into that cohort will cease, and the previous dose-level will be explored as the maximum tolerated dose (MTD). A total of 6 subjects will be enrolled into this lower-dose cohort, including previously enrolled subjects. Using the same criteria, lower-dose cohorts will be explored as the MTD until ≤ 1 of 6 subjects experience a DLT. d) Intermediate INXN-1001 dose levels may be recommended by the SRC (i) to advance the INXN-1001 dose in a smaller increment than originally planned, or (ii) to better determine the MTD. The intermediate dose level(s) will not exceed the higher of either a dose level in which >1 of 3 or 6 subjects experienced a DLT or the highest planned dose level (ie, Cohort 4, 160 mg/day). If 160 mg is the MTD and pharmacokinetic data suggests that higher dose(s) of INXN-1001 are indicated, then additional dose cohorts may be explored following an approved amendment of this protocol.

Duration of Subject Participation During Phase I and Phase II Group 1	Each subject's participation in this study will last approximately 7 months, including: • 30 day screening period. • 6 cycles (18 weeks) of study treatment. • Post-Treatment Safety Assessment visit performed 28 days after the last dose of INXN-1001. • Follow-Up Tumor Assessment visit performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. In addition, subjects who discontinue or complete study treatment in the absence of rapid clinical deterioration and without objective evidence of confirmed irPD should continue to be followed irPD has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.

SCHEDULE OF ASSESSMENTS FOR SUBJECTS IN PHASE I AND PHASE II GROUP 1

Activity	FOR PHASE I AND PHASE II GROUP 1 SUBJECTS									
	Screening[1]		ALL Cycles						Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Day 1	Day 2	Day 3 OR Day 4[27]	Day 7	Day 13	Day 15	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Clinical Assessments										
Informed Consent[2]	X									
Medical/Cancer History[3]	X									X[30]
Physical Exam[4]	X	X	X				X		X	
ECOG PS[5]	X		X						X	
Height	X									
Weight	X	X	X						X	
Vital Signs[6]	X	X	X	X	X[28]	X	X	X	X	
Adverse Events[7]	X	→	→	→	→	→	→	→	X	
Concomitant Med.[8]	X	→	→	→	→	→	→	→	→	X
Clinical Laboratory										
Pregnancy test[9]	X		X							
Hematology tests[10]	X		X[22]			X		X	X	
Serum Chemistry tests[11]	X		X[22]			X		X	X	
Urinalysis[12]	X		X[22]					X	X	
ECG[13]	X		X						X	
Serum Tryptase[14]			←	→	X	←	→			
Subject Registration[15]	X									
Study Drug Administration										
Intratumoral INXN-2001[23]			X							
Oral INXN-1001[24]			←	→	X	←	→			
Verify Adherence to INXN-1001 dosing[25]								X		

Activity	FOR PHASE I AND PHASE II GROUP 1 SUBJECTS									
	Screening[1]		Cycles 1, 3, and 6 ONLY						Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Day 1	Day 2	Day 3 & Day 4[27]	Day 7	Day 13	Day 15	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Ab responses[16]		X						X	X	
Cytokine profile[17]		X			X			X	X	
INXN-1001 PK[26]			X	X			X			

Activity	FOR SUBJECTS IN PHASE 1 DOSE ESCALATION						
	Screening[1]		Cycles 1, 3, and 6 ONLY			Post Treatment Safety Assessment Visit[28]	Follow-Up Tumor Assessment Visit[29]
	Day -28 to -7	Day -6 to -2	Cycle 1 Day 15±1	Cycle 3 Day 15±1	Cycle 6 Day 15±1	28±3 days post-INXN-1001 dose	35±7 days after PTSA visit
Tumor Response / Pharmacodynamics							
Imaging Studies/Tumor Assessment[18]	X			X		X	X
Digital photography[19]	X		X		X	X	X
PBMC[20]		X		X		X	X
Biopsy / FNA[31]		X	X	X		X	X
Tumor injected with INXN-2001			FNA	PBx	FNA (optional)	PBx	PBx
Non-Injected Tumor and/or Draining Lymph Node		PBx	FNA	PBx	FNA (optional)	PBx	PBx
Normal Skin Tissue		PBx or FNA				PBx or FNA	

NOTE: please refer to Table 2 Schedule of Assessments for description of all footnotes; footnote 31 is detailed below

31. Punch biopsies (PBx) or fine needle aspirate (FNA) samples of normal skin tissue, tumor(s) and/or associated tumor involved draining lymph nodes will be collected for *in vivo* assessment of transgene function and immunological activities, and other biological effects should be obtained at the time points indicated in the preceding table. Details of these procedures are described in the laboratory manual.

    PBx: Punch biopsy (≥4 mm in diameter); FNA: fine needle aspiration biopsy

    - At screening Day -6 to -2, punch biopsies should be obtained only after subject registration has been completed (i.e., after approval by sponsor for study participation) and before start of treatment.
    - Fine needle aspiration biopsies should be obtained on Day 15 after INXN-1001 administration.
    - Biopsies not required at Follow-Upup Tumor Assessment visit if ≤30 days since last biopsy.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ADA1001
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011

NOTE TO INVESTIGATORS

Amendment 1 dated 22 June 2011 will be used to conduct the study in place of any preceding version of this protocol.

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AMENDMENT 1

ADA1001

1. Summary and Rationale for Changes

- Implement clarifications and changes requested by a regulatory reviewer (U.S. Food & Drug Administration), including:
  - Exclude retreatment of subjects who experienced Grade 4 toxicity
  - Clarify retreatment criteria following a Grade 3 toxicity
  - Add day 8 physical exam and safety monitoring
  - Specify that SAEs will be reported in accordance with 21 CRF 312.32
- Update Medical Monitor contact information
- Increase the number of subjects who may be enrolled such that a single dose cohort may be expanded to up to 12 evaluable subjects in order to better define the safety, tolerability and preliminary antitumor activity of the study treatment
- Add a study objective and study procedures to assess adenovirus vector shedding in body fluids
- Correct minor typographical errors

2. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revision	Rationale for Change
Title Page and Footers.	**FROM:** 21 April 2011 **TO:** 22 June 2011	Update to current protocol version and date of amendment.
Title page	[redacted]	Updated medical Monitor information.

Section in Amended Protocol	Revision	Rationale for Change
Synopsis, Objectives; Section 6.1, Objectives	**FROM:** • Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells. **TO:** • Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells, and to assess adenovirus vector shedding in body fluids.	Add a study objective and study procedures to assess adenovirus vector shedding in body fluids.
Synopsis, No. of Subjects	**FROM:** 18 subjects (6 subjects per dose level cohort), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any. **TO:** 24 subjects (6 subjects per dose level cohort and up to 6 additional subjects enrolled at a single dose level at or below the MTD), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any.	Increase the number of subjects who may be enrolled such that a single dose cohort may be expanded to up to 12 evaluable subjects in order to better define the safety tolerability and preliminary antitumor activity of the study treatment.
Synopsis, Sample Size Determination; Section 6.2.2, Dose Escalation Procedure; Section 10.1, Determination of Sample Size	**FROM:** In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. If no DLTs are observed in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to the dose cohort so as to result in a total of 6 subjects for full analysis. **TO:** In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. If no DLTs are observed in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level. Based on a review of available safety and clinical response data, the Safety Evaluation Group may identify a single dose cohort to be expanded to a total of 12 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to the dose cohort so as to result in a total of 6 subjects for full analysis.	Increase the number of subjects who may be enrolled such that a single dose cohort may be expanded to up to 12 evaluable subjects in order to better define the safety tolerability and preliminary antitumor activity of the study treatment.
Section 4, Table 2: Schedule of Assessments	**FROM:** Day 8 **TO:** Day 8 ± 1 day **ADD:** 'X' for Physical Exam and Vital Signs within Day 8 column.	Add Day 8 physical exam per regulatory reviewer request. Add visit window to allow flexibility in visit scheduling.

Section in Amended Protocol	Revision	Rationale for Change
Section 4, Table 2: Schedule of Assessments	**ADD NEW STUDY PROCEDURE:** "Ad Vector Shedding" performed on study Day 2, 8 and 15 **ADD NEW FOOTNOTE:** 21. FOR CYCLE 1 ONLY: Optional sampling of body fluids may be performed to assess adenovirus vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Please refer to the laboratory manual for details regarding sample collection, processing and shipment. Note: Subjects may freely decline participating in all or any portion of this sampling without any effect on their study participation.	Add a study objective and study procedures to assess adenovirus vector shedding in body fluids.
Figure 3, legend	**FROM:** [redacted] [redacted] [redacted][9]	Correct typographical error (missing exponent).
Section 6.3, Definition and Management of Dose-Limiting Toxicity (DLT)	**FROM:** Based on the judgment of the Safety Evaluation Group, subjects who experienced a DLT will either be continued on treatment at the next lower dose of INXN-1001 or be withdrawn from the treatment and undergo early termination procedures. • If a subject experiences a DLT (other than a hypersensitivity or autoimmune reaction), the subject will either be continued on treatment with the next lower dose of INXN-1001 or will be withdrawn from treatment and undergo the Post-treatment safety assessment visit procedures **TO:** Subjects will be withdrawn from study treatment if they experience Grade 3 toxicity related to study drug that occurs after the initiation of treatment and that, upon review of the Safety Evaluation Group, is deemed to fit the definition of DLT. If, however, the patient has experienced a substantial benefit from treatment and the Safety Evaluation Group recommends continuation on the study, re-exposure to the experimental treatment may proceed on a case by case basis. Any subject who experiences a Grade 4 toxicity related to study drug will be withdrawn from study.	[redacted]
Section 7.3, Withdrawal of Subjects from Study Treatment and/or Study	**FROM:** • Unacceptable toxicity. • Study drug-related ≥Grade 3 hypersensitivity or autoimmune reaction. **TO:** • Any DLT meeting the withdrawal criteria described in Section 6.3. • Any treatment-related adverse event meeting withdrawal criteria as described in Section 8.2.6.	[redacted]
Section 8.2.6, Retreatment Criteria, Dose Delays and Modifications	**ADD:** Subjects who experience a treatment-related Grade 3 adverse event, other than nausea and/or vomiting in the absence of optimal treatment with anti-emetics, will be discontinued from study treatment. If, however, the patient has experienced a substantial	[redacted]

Section in Amended Protocol	Revision	Rationale for Change
	benefit from treatment and the Safety Evaluation Group recommends continuation on the study, re-exposure to the experimental treatment may proceed on a case by case basis. Subjects who experienced a Grade 4 treatment-related adverse event at any time during study treatment must be discontinued from study treatment.	
Section 8.4.2.2, Physical Examination	**FROM:** A complete physical exam will be performed at Screening and at the Post-treatment safety assessment visit. Symptom-directed physical exams will be performed at Day 1 and Day 15 of each cycle. **TO:** A complete physical exam will be performed at Screening and at the Post-treatment safety assessment visit. Symptom-directed physical exams will be performed at Days 1, 8 and 15 of each cycle.	Add Day 8 physical exam per regulatory reviewer request.
Section 8.4.2.13, Adenovirus Vector Shedding	Optional sampling of body fluids may be performed to assess adenoviral vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Subjects may freely decline participating in all or any portion of this sampling without any effect on their study participation.	[redacted]
Section 9.4, IND Safety Reports	**ADD:** ZIOPHARM will report all SAEs to the FDA in accordance with 21 CFR 312.32.	[redacted]
Throughout protocol;	**FROM:** CTCAE v4.0 **TO:** CTCAE v4.03	Clarify which version of CTCAE criteria will be used for this study.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101 (formerly ADA1001)
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012

NOTE TO INVESTIGATORS

Amendment 2 dated 13 January 2012 will be used to conduct the study in place of any preceding version of this protocol.

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1. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Throughout Protocol	Not applicable.	Numerous minor grammatical edits, clarifications, and other adjustments have been implemented throughout the protocol and will not be individually listed in this summary, but are indicated in the "track change" version of the study protocol. Examples include: • "Screening" was uncapitalized when not specifically referring to the Screening visit. • Visit windows were widened for some procedures. • Internal cross references to section numbers were updated, where appropriate.	Improve readability, apply minor updates implemented in other ZIOPHARM clinical protocols, and to and facilitate study conduct.
Throughout Protocol	Safety Evaluation Group	Safety Review Committee (SRC)	Update terminology.
Title Page, Page Headings and Footers.	Protocol ADA1001 22 June 2011	Protocol ATI001-101 (formerly ADA1001) 13 January 2012	Update to current protocol version and date of amendment.
Title Page	[redacted]	[redacted]	[redacted]

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 2 Abbreviations		*Added:* IEC Independent Ethics Committee PK Pharmacokinetics SRC Safety Review Committee	Add new abbreviations used amended text.
Section 3 Protocol Synopsis; Protocol Number	ADA1001	ATI001-101 (formerly ADA1001)	Update protocol number due to changes in ZIOPHARM's product numbering scheme.
Section 3 Protocol Synopsis; Objectives Section 6.1 Objectives	The secondary objectives are to: • Determine the recommended Phase II dose of the oral activator ligand for use with intratumoral injections of INXN-2001. • To obtain preliminary anti-tumor activity according to RECIST 1.1 criteria. • Evaluate the immunological effect of study treatment in terms of cellular and humoral immune responses, as well as other biological activities in the injected tumor(s), tumor-involved draining lymph nodes (if accessible) and in the peripheral circulation. • Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells, and to assess adenovirus vector shedding in body fluids.	The secondary objectives are to: • Inform the selection of an INXN-1001 dose(s) for further study in combination with INXN-2001. • Assess preliminary anti-tumor activity according to RECIST 1.1 criteria. • Assess anti-tumor response based on total measurable tumor burden. • Evaluate the immunological effect of study treatment in terms of cellular and humoral immune responses, as well as other biological activities in the injected tumor(s), tumor-involved draining lymph nodes (if accessible) and in the peripheral circulation. • Evaluate the extent of the uptake of INXN-2001 into tumor cells and tumor-infiltrating immune cells, and to assess adenovirus vector shedding in body fluids. • Assess the pharmacokinetics (PK) of INXN-1001 in subjects with unresectable stage III or IV melanoma.	Clarify secondary objective for INXN-1001 dose selection for further study (may be 1 or more doses). Add an objective to assess total measureable tumor burden. Add an objective to assess INXN-1001 PK.
Section 3 Protocol Synopsis; Study Drugs	Not applicable.	Added: Concurrent INXN-2001 and INXN-1001 dosing will be referred to as “study treatment” throughout this protocol.	Define “study treatment” as used throughout protocol.
Section 3 Protocol Synopsis; Number of Centers	Multi-center (approximately 2 to 5 centers)	Multi-center (approximately 5 to 10 centers)	Update number of centers planned for participation.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 3 Protocol Synopsis; Inclusion Criteria Section 7.1 Inclusion Criteria Section 7.2 Exclusion Criteria	Inclusion Criteria: 1. Males or females of all races ≥ 18 to ≤ 60 years of age, who have provided written informed consent prior to completing any study specific procedure. 2. Unresectable Stage III or Stage IV melanoma arising from a cutaneous, mucosal, subungal or unknown primary site. 5. Adequate bone marrow, liver, and renal function, as assessed by the following laboratory requirements: b. Lymphocytes > 1,000/ mm3 6. An expected survival of at least approximately 6 months. Exclusion Criteria: 1. Prior chemotherapy, immunotherapy, radiation therapy or any investigational agent within 30 days prior to the first dose of study drug. 2. Active infection requiring systemic antibacterial, antifungal, or antiviral therapy within 2 weeks of the first dose of study drug. 3. HIV-seropositive. 6. Previous treatment with any anti-tumor gene therapy.	Inclusion Criteria: 1. Males or females of all races ≥ 18 years of age, who have provided written informed consent prior to completing any study specific procedure. 2. Unresectable Stage III or Stage IV melanoma arising from any site other than ocular melanoma. 5. Adequate bone marrow, liver, and renal function, as assessed by the following laboratory requirements: b. Lymphocytes > 700/ mm3 6. An expected survival of at least 6 months. Exclusion Criteria: 1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. 2. Clinically significant infection requiring systemic antibacterial, antifungal, or antiviral therapy within 2 weeks of the first dose of study drug. 3. History of HIV infection. ~~6. Previous treatment with any anti-tumor gene therapy.~~ 6. Any medications that induce, inhibit or are substrates of CYP450 3A4 within 7 days prior to the first dose of study drug.	Revise eligibility criteria to increase the study population: • Remove upper age limit restriction • Simplify eligible melanoma primary sites of disease • Alleviate restriction on lymphocyte count per CDC standards • Remove restriction on prior gene therapy Clarify eligibility criteria to facilitate study conduct.
Section 3 Protocol Synopsis; Dose and Schedule	INXN-2001 should be injected into a different lesion at each cycle, and if the number of lesions is limited, the injections will be done in sequential rotation. One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001.	INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. Note: One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001.	Clarify INXN-2001 dosing when the number of accessible lesions is limited.
Section 3 Protocol Synopsis; Dose Escalation Plan	Three sequential dose escalation cohorts are planned, as described in Table 1.	Four sequential INXN-1001 dose escalation cohorts are planned, as described in Table 1.	A fourth dose cohort of INXN-1001 (160mg/day) was added in order to ensure the

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
			MTD is fully explored
Section 3 Protocol Synopsis; Table 1 Section 6.2.6 Dose Escalation Procedure; Table 1 (repeated)	Not applicable.	*Added footnote:* * Intermediate dose level(s) of INXN-1001 may be explored after review of all available safety and activity data, as may be decided by the SRC.	Add informational footnote (repeated from text).
Section 3 Protocol Synopsis; Dose Escalation Plan Section 6.2.6 Dose Escalation Procedure (formerly Section 6.2.2)	Dose escalation will not occur until the third subject in a cohort has completed the active treatment period (7 days of oral activator ligand) followed by 14 days of observation during the first treatment cycle. Following this, a meeting of the Safety Evaluation Group will be convened to review all reported ≥Grade 3 adverse events to determine if these meet dose-limiting toxicity (DLT) criteria. In addition, the Safety Evaluation Group will review the overall occurrence of adverse events and relevant laboratory data in each cohort to determine whether the MTD has been reached and to decide upon further subject enrollment. Subject enrollment and dose escalation will proceed using the following guidelines: a) If 0 of 3 subjects in a cohort experiences a DLT, then the next higher dose-level cohort may be enrolled. b) If 1 of 3 subjects in a cohort experiences a DLT, then up to a total of 6 subjects will be enrolled. c) If >1 of 3-6 subjects in a cohort experience a DLT, then enrollment into that cohort will cease, and the previous dose-level will be explored as the maximum tolerated dose (MTD), and a total of 6 subjects will be enrolled into that cohort. Using the same criteria, lower-dose cohorts will be explored as the MTD until ≤ 1 of 6 subjects experiences a DLT. d) Once a dose-level has been presumptively defined as the MTD using the above criteria, intermediate doses may be explored to further define the MTD, provided that ≤ 1 of 6 subjects experiences a DLT.	Dose escalation will not occur until the third subject in a cohort has completed the first treatment cycle (7 days of oral activator ligand followed by 14 days of observation). Following this, a meeting of the SRC will be convened to review all reported ≥Grade 3 adverse events to determine if these meet dose-limiting toxicity (DLT) criteria. In addition, the SRC will review the overall occurrence of adverse events and relevant laboratory data in each cohort to determine whether the MTD has been reached. Following this review, the SRC will advise on subject enrollment, dose escalation and/or cohort expansion using the following guidelines: a) If 0 of 3 subjects in a cohort experiences a DLT, then the next higher dose-level cohort may be enrolled. b) If 1 of 3 subjects in a cohort experiences a DLT, then up to 3 additional subjects will be enrolled into that cohort. c) If >1 of 3-6 subjects in a cohort experience a DLT, then enrollment into that cohort will cease, and the previous dose-level will be explored as the maximum tolerated dose (MTD). A total of 6 subjects will be enrolled into this lower-dose cohort, including previously enrolled subjects. Using the same criteria, lower-dose cohorts will be explored as the MTD until ≤ 1 of 6 subjects experiences a DLT. d) Intermediate INXN-1001 dose levels may be recommended by the SRC (i) to advance the INXN-1001 dose in a smaller increment than originally planned, or (ii) to better determine the MTD. The intermediate dose level(s) will not exceed the higher of either a dose level in which >1 of	Clarify dose escalation procedure.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
	During the dose escalation stage of the study, the initiation of dosing for each subject in any given dose cohort will be staggered by a minimum of seven days. However, subjects who will be enrolled after the MTD has been determined can be entered simultaneously.	3 or 6 subjects experienced a DLT or the highest planned dose level (i.e., Cohort 3, 100 mg/day). If 100 mg is the MTD and pharmacokinetic data suggests that higher dose(s) of INXN-1001 are indicated, then additional dose cohorts may be explored following an approved amendment of this protocol.	
Section 3 Protocol Synopsis; Definition of MTD	The highest dose studied in which a DLT has been seen in less than 2 out of 6 subjects (< 33%) during the first treatment cycle.	The highest dose of INXN-1001 studied in combination with INXN-2001 at which a DLT has been seen in less than 2 out of 6 subjects (< 33%) during the first treatment cycle. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered.	Clarify MTD definition.
Section 3 Protocol Synopsis; Definition of DLT	Dose-limiting toxicity (DLT) is defined as the occurrence of any study drug-related ≥Grade 3 adverse event occurring during the first treatment cycle, except nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics.	Dose-limiting toxicity (DLT) is defined as: • Any injection site reaction that includes phlebitis, ulceration, necrosis, severe tissue damage or where operative intervention is required. • Any local or systemic injection reaction that has life-threatening consequences, requires urgent intervention or results in death. • Any study drug-related ≥Grade 3 adverse event, except nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics.	Clarify and expand DLT definition to include local and systemic reactions.
Section 3 Protocol Synopsis; Duration of Subject Participation Section 11.4 Duration of The Study	Each subject's participation in this study will last approximately 4½ months, including: • 30 day screening period. • 3 cycles (9 weeks) of study treatment. • Post-treatment safety assessment visit performed 28 days after the last dose of INXN-1001.	Each subject's participation in this study will last approximately 5½ months, including: • 30 day screening period. • 3 cycles (9 weeks) of study treatment. • Post-treatment safety assessment visit performed 28 days after the last dose of INXN-1001. • Follow-up tumor assessment visit performed 35 ± 7 days after the Post-treatment safety assessment visit. In addition, subjects who discontinue or complete study treatment in the absence of rapid clinical deterioration and without objective evidence of confirmed immune-related	Add a Follow-up tumor assessment visit.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
		disease progression (irPD) should continue to be followed until immune-related disease progression has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.	
Section 3 Protocol Synopsis; Efficacy Evaluations	Not applicable.	*Added:* Additional assessment of anti-tumor activity will be explored based on total measurable tumor burden.[39] (Appendix 4)	Add central assessment of total tumor burden.
Section 3 Protocol Synopsis; Pharmacokinetic Evaluations	Not applicable.	*This row was added:* Pharmacokinetic Evaluations INXN-1001 pharmacokinetics will be evaluated in all subjects during cycle one.	Add INXN-1001 PK assessment.
Section 3 Protocol Synopsis; Sample Size Determination Section 10.1 Determination of Sample Size	This Phase I trial is being conducted primarily to provide descriptive safety and tolerability evaluations of intratumoral injections of INXN-2001 in subjects with Stage III or IV melanoma. No formal sample-size estimation was performed. The choice of the number of subjects was based on the standard Phase I 3 + 3 inter-cohort dose escalation design. Therefore, the maximum planned sample size will be 18 subjects if 6 subjects are assigned at each of the 3 dose levels. However, additional subjects may be added if exploration of intermediate dose level(s) of INXN-1001 is warranted. In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. In the event that the dose escalation phase does not result in 6 subjects in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level. Based on a review of available safety and clinical response data, the Safety Evaluation Group may identify a single dose cohort to be expanded to a total of 12 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be	No formal sample-size estimation was performed. The choice of the number of subjects was based on the standard Phase I 3+3 inter-cohort dose escalation design in which 3 to 6 subjects are enrolled into each dose level cohort. In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. In the event that the dose escalation phase does not result in 6 subjects in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level. Based on a review of available safety and clinical response data, the SRC may identify a single dose cohort to be expanded to a total of 12 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis. Given these considerations, a total of approximately 30 subjects may be enrolled into this study, including 6 subjects enrolled in each of the 3 planned dose level cohorts and up to 6 additional subjects enrolled at a single dose level at or below the MTD. Additional subjects may also be enrolled as	Clarify number of subjects planned for study.

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	added to the dose cohort so as to result in a total of 6 subjects for full analysis.	replacement subjects or to explore intermediate dose level cohorts, if any.	
Section 4, Table 2 Schedule of Assessments	Not applicable.	*Added procedures and visit to table:* Serum Tryptase INXN-1001 PK Follow-up Tumor Assessment Visit	Add safety assessment (serum tryptase). Add INXN-1001 PK assessment. Add a Follow-up tumor assessment visit.
Section 4, Table 2 Schedule of Assessments; Footnotes	Not applicable.	*New footnotes added:* 14. Serum tryptase level will be obtained upon the occurrence of a suspected injection and/or hypersensitivity reaction. Otherwise, this test is not required at any other time. 23. FOR CYCLE 1 ONLY: Subjects will undergo an INXN-1001 pharmacokinetic (PK) assessment. Blood samples for INXN-1001 PK analysis should be obtained on the following days and timepoints: Day 1: Pre-dose (< 30 minutes prior to INXN-1001 dosing) 1-2 hours post INXN-1001 dosing 3-8 hours post INXN-1001 dosing Day 2: Pre-dose (< 30 minutes prior to INXN-1001 dosing) Day 7: 1-2 hours post INXN-1001 dosing 3-8 hours post INXN-1001 dosing Day 8 OR Day 9: anytime >24 hours post-Day 7 INXN-1001 dose 27. Day 1 clinical laboratory assessments may be performed within 2 days prior to the Day 1 clinic visit. Clinical laboratory tests drawn for analysis on Day 15 of each cycle may be used for determining if the retreatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration (see Section 8.2.6). 30. A Follow-up tumor assessment visit will be performed 35 ± 7 days after the Post-treatment safety assessment visit. This	Add safety assessment (serum tryptase). Add INXN-1001 PK assessment. Add cross reference to retreatment criteria (Section 8.2.6). Add a Follow-up tumor assessment visit.

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		assessment is required for all subjects, including those with prior objective evidence of disease progression. Visit procedures will include a tumor response assessment per RECIST v1.1 guidelines (see footnote 16), digital photography of all visible tumors (see footnote 17), and documentation of any anti-cancer treatments received since the previous tumor assessment. Subjects without objective evidence of disease progression at the Follow-up tumor assessment visit should continue to have tumor assessments performed at 8-10 week intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first. 31. Interim cancer history information will include documentation of any anti-cancer treatments received since the previous tumor assessment.	
Section 4, Table 2 Schedule of Assessments; Footnotes	7. AEs/SAEs will be collected after the start of INXN-1001 dosing through the Post-treatment safety assessment visit. AEs/SAEs that occur after the subject has signed informed consent and prior to INXN-1001 dosing will be entered into the medical history. However, study drug-related AEs/SAEs that are ongoing at the time of the Post-treatment safety assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor). 8. Concomitant medication information, including blood products, vitamins and other supplements will be collected for the time period beginning 28 days prior to the first dose of study drug, through the Post-treatment safety assessment visit. 15. Appropriate cancer staging procedures should be performed during Screening, including chest and abdominal CT or MRI scans. For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-	7. Monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. AEs/SAEs that occur following informed consent until the Post-treatment safety assessment must be recorded on the AE CRF; AEs/SAEs that occur prior to informed consent should be added to the medical history CRF. Study drug-related AEs/SAEs that are ongoing at the time of the Post-treatment safety assessment visit should be followed until resolution, return to baseline, they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor), or they can be attributed to other than the study drug or other than study conduct. SAEs considered to be related to study drug that occur beyond 30 days after the last dose must also be reported. In addition, all SAEs must be reported by the investigator or designee within 24 hours of becoming aware of the event, from the time of informed consent through 30 days after the last dose of study drug, regardless of the initiation of any new anti-cancer therapy. SAEs considered to be related to study drug that occur beyond 30 days after the last dose	Update Adverse Event sections to be consistent with changes made in other ZIOPHARM protocols. ConMeds will be collected through Follow-up tumor assessment visit. Revise for consistency within study protocol. Revise text for clarity. Add a Follow-up tumor assessment visit. Add INXN-1001 PK assessment.

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	2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). Disease sites are to be assessed throughout the study using the same method(s) used at Screening. All subjects will have tumor assessments performed within 2 weeks prior to the Post-treatment safety assessment visit. The Investigator will evaluate response to therapy according to RECIST 1.1 guidelines. Subjects who discontinue or complete study treatment without objective evidence of disease progression should continue to have tumor assessments performed at 8 10 week intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first. Details regarding response assessments will be provided in the investigator's study files. 21. FOR CYCLE 1 ONLY: Optional sampling of body fluids may be performed to assess adenovirus vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Please refer to the laboratory manual for details regarding sample collection, processing and shipment. Note: Subjects may freely decline participating in all or any portion of this sampling without any effect on their study participation 26. Post-treatment assessment visit is to be performed 28 ± 2 days after the last dose of INXN-1001 or at the time of early withdrawal from study treatment. Subjects with ongoing study drug-related AEs/SAEs should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor). Subjects without objective evidence of disease progression should continue to have tumor assessments performed at 8 - 10 weeks intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first.	must also be reported. 8. Concomitant medication information, including blood products, vitamins and other supplements will be collected for the time period beginning 28 days prior to the first dose of study drug, through the Follow-up tumor assessment visit. (~~15~~) 16. Appropriate cancer staging procedures should be performed during screening, including chest and abdominal CT or MRI scans. PET scans may be accepted in place of CT or MRI scans following consultation and agreement by the ZIOPHARM Medical Monitor. For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). Disease sites are to be assessed throughout the study using the same method(s) used at screening. All subjects will have at least 2 tumor response assessments performed following the initiation of study treatment: • At the Post-treatment safety assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment. • At the Follow-up tumor assessment visit (35 ± 7 days following the Post-treatment safety assessment visit). The Investigator will evaluate response to therapy according to RECIST v1.1 guidelines. NOTE: As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, anti-tumor response will be explored by measuring total tumor burden over time. (see Appendix 4) (~~21~~)22. FOR CYCLE 1 ONLY: Subjects will be asked to provide samples of body fluids to assess any potential adenoviral vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen.	

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		Subjects will sign a consent form if they agree, but may freely decline to participate in all or any portion of this sampling without effect on their core study participation or prejudice to their future care. Please refer to the laboratory manual for details regarding sample collection, processing and shipment. (~~26~~) 29. Post-treatment safety assessment visit will be performed 28 ± 3 days after the last dose of INXN-1001 or at the time of early withdrawal from study treatment. Note: imaging studies performed for the tumor response assessment may be completed within 2 weeks prior to the Post-treatment safety assessment visit. Subjects with ongoing study drug-related AEs/SAEs should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor) , or they can be attributed to other than the study drug or other than study conduct. Subjects found to have residual viral particles or DNA in tissue or blood samples may be requested to return to the clinic at monthly intervals for further testing until clearance has been documented.	
Section 5.1 Disease Background (Melanoma)	… Ipilimumab, an anti-CTLA-4 mono-clonal antibody used to overcome or reverse T cell suppression and thereby potentiate an antitumor T-cell response, was evaluated in a Phase III study in patients with unresectable Stage III and IV melanoma. The patients were randomized to receive ipilimumab plus gp100 (403 patients), ipilimumab alone (137 patients), or a gp100 vaccine alone (136 patients). The median overall survival with ipilimumab alone was 10.1 months (hazard ratio of 0.66 for death in comparison with gp100 alone-; p=0.003). The most common drug-related adverse events were immune related. These occurred in approximately 60% of patients treated with ipilimumab and 32.5% in patients treated with gp100. The frequency of Grade 3 and 4 immune-related events was 10 to 15% in the ipilimumab group. These immune related adverse events most often affected the skin and the	… Ipilimumab, an anti-CTLA-4 mono-clonal antibody used to overcome or reverse T cell suppression and thereby potentiate an antitumor T-cell response, was approved by the FDA for treatment of unresectable Stage III and IV melanoma on the basis of a Phase III trial which demonstrated an improvement in median overall survival. In that study, there were 14 deaths related to the study drugs and 7 were associated with immune related adverse events.[3] Other immunotherapies for the treatment of melanoma are also in various stages of development, including OncoVEX^{GM-CSF}, an oncolytic herpes simplex virus encoding GM-CSF.	Informational update.

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	gastrointestinal tract (most commonly diarrhea) and also included hypophysitis and thyroiditis. The immune related events required treatment with high doses of corticosteroids in order to control these clinically significant safety problems. There were 14 deaths related to the study drugs and 7 were associated with immune related adverse events.[3]		
6.2.2 Treatment Parameters and Duration (formerly Section 6.2.3)	All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 3-week cycle. The subjects will also receive a single daily oral dose of INXN-1001 for 7 consecutive days starting on Day 1 of each cycle. The INXN-1001 dose assigned to any given subject will progress as per the dose escalation schedule above. INXN-2001 should be injected into a different lesion at each cycle, and if the number of lesions is limited, the injections should be done in sequential rotation. One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001. Subjects may receive up to 3 cycles of study treatment. Because tumor nodules may enlarge due to the anticipated induction of an inflammatory response, subjects with disease progression (per RECIST criteria) may still complete the 3 treatment cycles, unless the progression is clinically significant, e.g. the ECOG performance status increases during the protocol to ≥ 2.	All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 3-week cycle. The subjects will also receive a single daily oral dose of INXN-1001 for 7 consecutive days starting on Day 1 of each cycle. The INXN-1001 dose assigned to any given subject will be determined according to the dose escalation schedule (Section 6.2.6). Subjects may receive up to 3 cycles of study treatment. Because tumor nodules may enlarge due to the anticipated induction of an inflammatory response, subjects with disease progression (per RECIST criteria) may still complete the 3 treatment cycles, unless the progression is clinically significant, e.g., development of new metastases or an ECOG performance status that increases during study treatment to ≥ 2.	Clarify text.
6.2.3 Safety Review Committee (SRC) (formerly Section 6.2.4 Safety Evaluation Group)	A Safety Evaluation Group comprised of the Medical Monitor, Principal Investigators and sponsor representatives, will hold periodic teleconferences to evaluate the safety and treatment status of all subjects.	A SRC comprised of the Medical Monitor, Principal Investigators and sponsor representatives, will hold periodic teleconferences to evaluate the safety and treatment status of all subjects. The SRC will review and assess the safety data at the completion of each dose cohort as described in Section 6.2.6, and at any other time as needed. The following data will be reviewed at each SRC meeting to determine whether to proceed to a subsequent cohort: physical examination and vital signs; medical history; complete blood count with differential; serum chemistry panel including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin; lactate	Clarify dose escalation procedure. Provide additional information regarding SRC review meetings and procedures.

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		dehydrogenase (LDH); albumin; serum tryptase level; ECG; cytokine levels; and adverse event and concomitant medication records. Additional information will be reviewed as necessary. A written summary documenting the results and recommendations of each review will be provided to the Investigator(s) and maintained on file with the sponsor. Additional sub-Investigators and personnel may participate in reviews as indicated. Following a cohort review, the SRC may recommend proceeding with enrollment in the next dose cohort, enrolling additional subjects in the current cohort, dropping back to a lower cohort, exploring an alternate dose level, or not enrolling any additional subjects. The dose escalation and enrollment guidelines outlined in Section 6.2.6, as well as the study stopping rules outlined in Section 6.2.7, will be used as the basis for these assessments. While the SRC is expected to reach a consensus opinion regarding any premature discontinuation or significant modification of the study, the sponsor may independently stop the study.	
6.2.4 Definition and Management of Dose-Limiting Toxicity (DLT) (formerly Section 6.3)	Dose-limiting toxicity (DLT) is defined as the occurrence of any study drug-related ?Grade 3 adverse events occurring during the first treatment cycle, except nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics. If DLTs have been observed in >1 of the 6 subjects in a cohort during the first treatment cycle, the MTD will have been exceeded and further use of that dose will be discontinued in all subjects, and the next lower dose cohort will be assessed as to whether it meets the definition of the MTD. No further use of a dose of INXN-1001 above the MTD will be allowed. Subjects will be withdrawn from study treatment if they experience Grade 3 toxicity related to study drug that occurs after the initiation of treatment and that, upon review of the Safety Evaluation Group, is deemed to fit the definition of DLT. If, however, the patient has experienced a substantial benefit from treatment and the Safety Evaluation Group	Dose-limiting toxicity (DLT) is defined as: • Any injection site reaction that includes phlebitis, ulceration, necrosis, severe tissue damage or where operative intervention is required. • Any local or systemic injection reaction that has life-threatening consequences, requires urgent intervention or results in death. • Any study drug-related ≥Grade 3 adverse event, except nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics. If DLTs have been observed in >1 of the 3-6 subjects in a cohort during the first treatment cycle, the MTD will have been exceeded and further use of that dose will be discontinued in all subjects, and the next lower dose cohort will be assessed as to whether it meets the definition of the MTD.	Clarify and expand DLT definition to include local and systemic reactions. Add requirement that the Medical Monitor be notified of potential DLTs.

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	recommends continuation on the study, re-exposure to the experimental treatment may proceed on a case by case basis. Any subject who experiences a Grade 4 toxicity related to study drug will be withdrawn from study.	The Medical Monitor should be immediately notified of any potential DLT. Subjects will be withdrawn from study treatment if they experience an injection site reaction that meets DLT criteria or other Grade 3 toxicity related to study drug that, upon review of the SRC, is deemed to be a DLT. If, however, the subject has experienced a substantial benefit from treatment and the SRC recommends continuation on the study, re-exposure to the experimental treatment may proceed at a lower dose of INXN-1001 on a case by case basis. Any subject who experiences a Grade 4 toxicity related to study treatment will be withdrawn from study.	
6.2.5 Definition of Maximum Tolerated Dose (MTD) (formerly Section 6.4)	The MTD will be defined as the highest dose studied in which a DLT has been seen in less than 2 out of 6 subjects (< 33%) during the first treatment cycle. After the MTD has been determined based on DLTs during the first treatment cycle within a cohort, the MTD will remain the same even if additional DLTs are determined during the second or third treatment cycle.	The MTD will be defined as the highest dose of INXN-1001 studied in combination with INXN-2001 at which a DLT has been seen in less than 2 out of 6 subjects (< 33%) during the first treatment cycle. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered.	Clarify MTD definition.
Section 6.2.7 Study Stopping Rules	Not applicable.	*The following section was added:* 6.2.7 Study Stopping Rules Rules for stopping dose escalation due to DLTs observed during the first cycle of study treatment are outlined in Section 6.2.6. In the event that a given dose level cohort is determined to have exceeded the MTD, then further use of that dose will be discontinued for all subjects as noted in Section 6.2.4. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered. Stopping rules for toxicities that occur from Day 1, Cycle 2 through the Post-treatment follow-up visit will apply as follows: • If an adverse event can be definitely shown to be unrelated to study treatment (e.g., motor vehicle accident, bee sting, elective surgery), that specific toxicity will not be considered in a stop vs. dose escalation decision.	Clarify dose escalation procedure. Add study stopping rules to enhance subject safety.

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		• Recruitment in the current cohort will not be stopped for Grade 2 (NCI CTCAE v.4.03) non-dose limiting toxicities, irrespective of the number of events or number of subjects. • Recruitment in the current cohort will be stopped for any Grade 3 or 4 toxicity, unless the adverse event is clearly and incontrovertibly due to extraneous causes. All data will be reviewed by the Investigator, the Investigator's IRB, ZIOPHARM Medical and Regulatory Staff, and the SRC to determine if the study should proceed and if/what changes must be implemented. • In addition to stopping rules for dose escalation, patient enrollment into the study will be halted in the event of any study drug-related death. In this case, patient enrollment will only resume following review and approval of the SRC. For toxicity grading based on laboratory tests, an abnormality must be confirmed (when appropriate) by repeat testing. For any suspected injection and/or hypersensitivity reaction, a serum tryptase level will be measured.	
Section 7.3 Withdrawal of Subjects from Study Treatment and/or Study	A subject **may** withdraw (or be withdrawn) from the study treatment prematurely for any of the following reasons: • Principal investigator (PI) determines further participation is not in subject's best interest. • Clinically significant disease progression.	A subject **may** withdraw (or be withdrawn) from the study treatment prematurely for any of the following reasons: • Principal investigator (PI) determines further participation is not in subject's best interest. • Clinically significant disease progression. **NOTE:** In the absence of rapid clinical deterioration, disease progression should be confirmed by a repeat, consecutive assessment no less than 4 weeks from the date first documented to ensure that more slowly declining tumor burden in response to therapy is not missed.	Clarification for the need of a confirmatory progression scan.
Section7.4 Replacement of Subjects	Subjects who are withdrawn from study treatment prior to completing Cycle 1 dosing for reasons other than toxicity may be replaced so that a full cohort of subjects completes Cycle 1 safety evaluations. In addition, should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to that dose cohort	Subjects who are withdrawn from study treatment prior to completing Cycle 1 dosing for reasons other than toxicity may be replaced so that a full cohort of subjects completes Cycle 1 safety evaluations. In addition, should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to that dose cohort so as to result in a total of at least three subjects for full	Clarify that replacement of subjects also applies to cohorts of 3 subjects.

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	so as to result in a total of six subjects for full analysis.	analysis.	
8.2.3 Treatment Regimen	INXN-2001 will be given as intratumoral injections of [redacted] in 0.5 mL per treatment. If no accessible tumor is available, a tumor-involved draining lymph node will be injected. The injections will be administered on the first day of each cycle throughout the study.	INXN-2001 will be given as intratumoral injections of [redacted] in 0.5 mL per treatment. Following Cycle 1, if no accessible lesion for INXN-2001 injection is present (e.g., due to complete resolution), then INXN-2001 should be injected into a draining lymph node of a previously accessible lesion. The injections will be administered on the first day of each cycle throughout the study.	Clarify INXN-2001 dosing when the number of accessible lesions is limited.
8.2.5.3 Preparation and Administration of INXN-2001	INXN-2001 [redacted] should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or tumor-involved lymph node, at approximately 0.5 cm apart three dimensionally, to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. The injection should be given 3 hours ± 30 minutes after the INXN-1001 dose. A detailed description and photograph of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions should be documented. Note: One of the minimum of two accessible lesions (per the eligibility criteria) will not be injected as that lesion will be used to evaluate the systemic effect of INXN-2001. If ≥2 accessible lesions are present (not including the lesion that will not be injected), INXN-2001 will be injected into a different lesion at each cycle, to the extent feasible. If the number of lesions is limited, the injections will be done in sequential rotation. After the first cycle, should the accessible tumor not support the INXN-2001 injection volume, another tumor should be injected with the remaining volume to ensure that all subjects receive [redacted]. If an accessible lesion is not present, INXN-2001 will be injected into a draining lymph node of a previously accessible lesion.	INXN-2001 [redacted] will be administered as an intratumoral injection 3 hours (± 30 minutes) after oral INXN-1001 dosing on Day 1 of each cycle. INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. Note: One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001. The INXN-2001intratumoral administration should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or tumor-involved lymph node, at approximately 0.5 cm apart three dimensionally, to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. A detailed description and photograph of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions should be documented. Should the tumor selected for injection not support the entire INXN-2001 injection volume, another tumor should be injected with the remaining volume to ensure that all subjects receive [redacted] If another tumor is not available, then the remaining volume should be injected into a draining lymph node of the injected tumor. Following Cycle 1, if no	Clarify INXN-2001 dosing procedure.

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		accessible lesion for INXN-2001 injection is present (e.g., due to complete resolution), then INXN-2001 should be injected into a draining lymph node of a previously accessible lesion.	
Section 8.2.6 Retreatment Criteria, Dose Delays and Modifications	Subjects may receive up to 3 cycles of study drug treatment. In order to receive each repeat treatment cycle, all treatment-related adverse events must have resolved to Grade 1 or baseline. … Subjects who experience a DLT will be managed as described in Section 7.3.	Subjects may receive up to 3 cycles of study drug treatment. In order to receive each repeat treatment cycle, all treatment-related adverse events must have resolved to Grade 1 or baseline and the following laboratory criteria must be met: • Hemoglobin ≥ 9 g/L • Lymphocytes > 700/ mm^3 • Neutrophils ≥ 1,500/ mm^3 • Platelets ≥ 100,000/ mm^3 • Serum creatinine ≤ 1.5 x ULN • AST and ALT ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5×ULN. • Total bilirubin < 1.5 x ULN • INR and PTT <1.5 x ULN, if not therapeutically anticoagulated. Clinical laboratory tests drawn for analysis on Day 15 of each cycle may be used for determining if the retreatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration. … Subjects who experience a DLT will be managed as described in Section 6.2.4. Subjects who experience an injection site reaction should be managed as described in Section 8.2.7.	Add retreatment criteria to enhance subject safety.
Section 8.2.7 Severity Grading and Management of Injection Site Reactions	Not applicable.	*This section was added:* 8.2.7 Severity Grading and Management of Injection Site Reactions Injection of a biologic agent carries a potential risk of injection site reaction which is characterized as an intense reaction (usually immunologic) at or near the site of injection. Injection reactions will be graded according to the National Cancer	Add section addressing management and grading of potential injection site reactions as an additional safety measure.

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		Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Table 3 outlines the injection site reaction grading system, associated symptoms, and the recommended actions to be taken. For any injection reaction, a serum tryptase level will be measured. Reactions at the INXN-2001 injection site that occur within 24 hours after the injection and are attributed to INXN-2001 will be considered to be injection related AEs. < Table 3 > As with all AEs, injection-related signs or symptoms which, in the Investigator's judgment, are not related to immunologic reactions (e.g., dyspepsia, urinary frequency, etc.) should be recorded and graded as AEs according to NCI CTCAE v.4.03 criteria in the CRF. Study stopping rules will not apply to a specific event if it is clearly unrelated to INXN-2001 injection (e.g. accidental trauma). If a AE associated with injection cannot be definitely shown to be unrelated to INXN-2001, then study stopping rules will apply.	
8.2.8 Prophylactic Antipyretic and/or Analgesic Administration (formerly Section 8.2.7 Prophylactic Acetaminophen Administration)	Treatment-related fever during Cycle 1 may be treated with acetaminophen. It is recommended that subjects who experience treatment-related fever during Cycle 1 be administered 700 mg (2 x 350 mg) of acetaminophen prior to injection of INXN-2001 in subsequent cycles. In addition, if the subject needs additional doses of acetaminophen, he/she should be instructed to take one or two 350 mg tablets every 6 hours as needed for symptoms (pain at injection site, fever, etc.). If the subject needs to continue taking the acetaminophen for longer than two days, he/she should be instructed to contact the clinical site.	The use of antipyretics and/or analgesics is allowed anytime during study treatment, as indicated, including prophylactic administration. Since fever and flu like symptoms are commonly experienced following adenoviral vector administration it is strongly recommended that subjects be treated with prophylactic antipyretic and/or analgesic medications prior to study drug administration. For those subjects who experience treatment related symptoms (e.g. fever, headache, chills, etc.) during Cycle 1, prophylactic antipyretic and/or analgesic medications must be given prior to study drug administration in subsequent cycles. Please refer to Appendix 3 for a recommended regimen for the prophylactic administration of antipyretics and/or analgesics.	Clarify recommendation on the use of prophylactic medications.
Section 8.2.9 Disposition of Unused Study	All unused study drug must be returned to the sponsor or destroyed on site in accordance with applicable site facility and US Occupational Safety and Health Administration (OSHA) procedures after a full accountability has been documented.	All unused study drug should be destroyed on site in accordance with applicable site facility and US Occupational Safety and Health Administration (OSHA) procedures after a full accountability has been documented. Any on-site	Clarify that unused study drug should be destroyed on site (rather than being returned to

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Drug (formerly Section 8.2.8)	Any on-site destruction of unused study drug must be documented and the records maintained in the Investigator's study file.	destruction of unused study drug must be documented and the records maintained in the Investigator's study file.	the sponsor).
Section 8.3 Concomitant Therapy	Permitted: • Subjects may receive standard treatments, including palliative and supportive care for any underlying illness. • Subjects should be cautioned to avoid, if possible, medications, foods or drinks that induce, inhibit, or are substrates of the CYP450 3A4 pathway. See Appendix 2 for examples. Not Permitted: • Subjects may not receive other investigational or approved cancer therapy (chemotherapy, etc.) while receiving study treatment.	Permitted: • Subjects may receive standard treatments, including palliative and supportive care for any underlying illness with the exception of palliative radiotherapy which is NOT permitted. • Antidiarrheal therapy is permitted for study drug-induced diarrhea. • Antiemetics are permitted for study drug-induced nausea and vomiting. • Treatment with vitamin/mineral supplements is acceptable provided that they do not interfere with study endpoints, in the opinion of the investigator. Not Permitted: • Subjects may not receive medications, foods or drinks that induce, inhibit, or are substrates of the CYP450 3A4 pathway within 7 days prior to the first dose of study drug through 96 hours after their last dose of INXN-1001. See Appendix 2 for examples. • Subjects may not receive any other investigational agent or anti-cancer therapy (chemotherapy, radiotherapy, etc.) while receiving study treatment.	Clarify that concurrent prophylactic radiotherapy is not permitted during study treatment. Prohibition of medications, foods or drinks that induce, inhibit, or are substrates of the CYP450 3A4.
Section 8.4.2.6 Adverse Events	Monitoring and recording of adverse events (AEs) will be conducted throughout the study. AEs, including serious adverse events (SAEs), will be captured on the CRFs from the start of INXN-1001 dosing (Cycle 1, Day 1) through the Post-treatment safety assessment visit. However, study drug-related AEs/SAEs that are ongoing at the time of the Post-treatment safety assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor). Definitions, documentation, and reporting of	Monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. AEs/SAEs that occur following the first dose of study drug until the Post-treatment safety assessment must be recorded on the AE CRF; AEs/SAEs that occur following informed consent, but prior to the first dose of study drug, should be added to the medical history CRF. Study drug-related AEs/SAEs that are ongoing at the time of the Post-treatment safety assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized (and	Update Adverse Event sections to be consistent with changes made in other ZIOPHARM protocols.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
	AEs are described in Section 10.	following consultation and agreement by the ZIOPHARM Medical Monitor). In addition, all SAEs must be reported by the investigator or designee via a paper or electronic SAE Report form within 24 hours of becoming aware of the event, from the time of informed consent through 30 days after the last dose of study drug, regardless of the initiation of any new anti-cancer therapy. SAEs considered to be related to study drug that occur beyond 30 days after the last dose must also be reported. Definitions, documentation, and reporting of AEs and SAEs are described in Section 10.	
Section 8.4.2.12 Serum Tryptase	Not applicable.	*This section was added:* 8.4.2.12 Serum Tryptase A serum tryptase level will be obtained upon the occurrence of a suspected injection and/or hypersensitivity reaction.	Add safety assessment (serum tryptase).
Section 8.4.2.13 Immune Response Analysis (formerly Section 8.4.2.12)	… Serum IL-12 and IFN-γ will be assessed in as close to real time as possible for Day 1 through Day 4 timepoints, and clinically significant increases will be discussed by the Medical Monitor and Principal Investigator.	… One or more serum cytokines may be assessed in as close to real time as possible for one or more time points in a dosing cycle at the request of the Medical Monitor or other sponsor representative(s).	Remove requirement for "real time" cytokine analyses to facilitate study conduct.
Section 8.4.2.14 Adenovirus Vector Shedding (formerly Section 8.4.2.13)	Optional sampling of body fluids may be performed to assess adenoviral vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Subjects may freely decline participating in all or any portion of this sampling without any effect on their study participation.	Subjects will be asked to provide samples of body fluids to assess any potential adenoviral vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Subjects will sign a consent form if they agree, but may freely decline to participate in all or any portion of this sampling without effect on their core study participation or prejudice to their future care.	Minor clarification of text.
Section 8.4.3 INXN-1001 Pharmacokinetic Assessment	Not applicable.	*This section was added:* 8.4.3 INXN-1001 Pharmacokinetic Assessment Subjects will undergo an INXN-1001 pharmacokinetic (PK) assessment during cycle one of their study treatment. Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day 8 or 9 as defined in the	Add INXN-1001 PK assessment.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
		Schedule of Assessments (see Table 2)..	
Section 8.4.4 Efficacy Assessments (formerly Section 8.4.3)	All subjects will have tumor assessments performed within 2 weeks prior to the Post-treatment safety assessment visit. The Investigator will evaluate each subject for response to therapy according to RECIST 1.1 guidelines.1 Subjects who discontinue or complete study treatment without objective evidence of disease progression should continue to be followed for response at 8 -10 weeks intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first. … Details regarding response assessments and photographs will be provided in the investigator's study files.	All subjects will have tumor assessments performed within 2 weeks prior to the Post-treatment safety assessment visit and again at the Follow-up tumor assessment visit. The Investigator will evaluate each subject for response to therapy according to RECIST 1.1 guidelines.1 Subjects without objective evidence of disease progression at the Follow-up tumor assessment visit should continue to have tumor assessments performed at 8-10 week intervals until disease progression has been documented or an alternate anti-cancer therapy has been initiated, whichever occurs first. … Details regarding photographic methodology will be provided in the Photography Operations Manual As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, the sponsor will be analyzing anti-tumor response by assessing total tumor burden over time. Please refer to Appendix 4 for a description of this "immune-related Response Criteria" (irRC).	Add a Follow-up tumor assessment visit. Clarification of provided reference materials. Addition of central assessment of Total tumor burden.
Section 9 Adverse Events	*Entire Section*	*Entire Section*	Entire section replaced with new ZIOPHARM Template language.
Section 10 Statistical Procedures	Data from this study will be analyzed and included in an interim report after the last subject has completed the Post-treatment safety assessment visit. Additional data collected from subjects who are assessed for tumor response every 8-10 weeks will be provided as an addendum to the study report.	Data from this study will be analyzed and included in a clinical study report that will be prepared after the last subject has completed the Post-treatment safety assessment visit. Additional data collected from subjects who have not progressed by the Post-treatment safety assessment visit and are continuing to be followed for tumor response at the time of the final data cut-off date will be presented in an addendum to the study report.	Clarify text.
Section 10.1.2 Populations for Analysis	• Activity Evaluable Population: All subjects in the Safety Population who have at least 1 post-baseline response evaluation.	• Activity Evaluable Population: All subjects who received at least 1 dose of INXN-2001 and INXN-1001, and have at least 1 post-screening response evaluation.	Clarify definition of Activity Evaluable Population
Section 10.3	Data will be summarized by dose cohort.	Data will be summarized by dose cohort based on the actual	Clarify text.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Statistical Methods		dose of INXN-1001 received on Day 1, Cycle 1.	
Section 10.3.3 **Efficacy Analyses**	The Activity Evaluable Population will be used for the analyses of efficacy data based on the dose of INXN-1001 received. Following completion of the study, best response will be determined for each subject in accordance with RECIST v.1.1 guidelines and the objective response rate presented for each dose cohort. Progression-free survival and durability of response will be determined using Kaplan-Meier methodology.	The Activity Evaluable Population will be used for the analyses of efficacy data based on the dose of INXN-1001 received. Following completion of the study, best response will be determined for each subject in accordance with RECIST v.1.1 guidelines and the objective response rate presented for each dose cohort. Progression-free survival and durability of response will be determined using Kaplan-Meier methodology. As a supplement to the standard RECIST v1.1 guidelines established to evaluate anti-tumor responses to chemotherapeutic agents, the sponsor will be analyzing anti-tumor response by assessing total tumor burden over time. Please refer to Appendix 4 for a description of this "immune-related Response Criteria" (irRC).	Addition of central assessment of Total tumor burden.
Section 10.3.5 INXN-1001 Pharmacokinetics	Not applicable.	*This section was added:* 10.3.5 INXN-1001 Pharmacokinetics INXN-1001 PK parameters to be determined will include, but is not limited to, the maximum concentration (Cmax), time to maximum concentration (Tmax), half-life ($t_{1/2}$), area-under-the-concentration time curve (AUC), volume of distribution (Vd), and clearance (CL). Where possible, descriptive statistics of the PK parameters will be provided; individual subject INXN-1001concentrations, actual sampling times, and PK parameters will be listed.	Add INXN-1001 PK assessment for subjects receiving medications relevant to CYP450 3A4.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 11.6 Institutional Review Board	This protocol and the study informed consent form must be reviewed and approved by the IRB or IEC prior to the start of the study and a copy of the approval letter supplied to ZIOPHARM.	This protocol and the study informed consent form must be reviewed and approved by the Institutional Biosafety Committee (where applicable) and IRB/IEC prior to the start of the study and a copy of the approval letter supplied to ZIOPHARM.	Add mention that review and approval of Institutional Biosafety Committee is also required, where applicable.
Appendix 3 SUGGESTED REGIMEN FOR ANTIPYRETIC AND/OR ANALGESIC PROPHYLAXIS	*Not applicable*	*Entire new appendix*	Addition of a recommended antipyretic regimen in order to prophylax patients prior to dosing with INXN-1001
Appendix 4 IMMUNE-RELATED RESPONSE CRITERIA (irRC)	*Not applicable*	*Entire new appendix*	Description of the response criteria to be used to measure total tumor burden.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101 (formerly ADA1001)
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012 Amendment 3: 20 April 2012

NOTE TO INVESTIGATORS

Amendment 3 dated 20 April 2012 will be used to conduct the study in place of any preceding version of this protocol.

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1. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Throughout Protocol	Not applicable.	A few minor grammatical edits, clarifications, and other adjustments have been implemented throughout the protocol and will not be individually listed in this summary, but are indicated in the "track changes" version of the study protocol.	Improve readability.
Title Page, Page Headings and Footers.	Amendment 2: 13 January 2012	Amendment 3: 19 April 2012	Update to current protocol version and date of amendment.
Title Page	[redacted]	[redacted]	Update Sponsor contact information.
Section 3 Protocol Synopsis; Study Design	Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in duration. **There will be at least 7 days between the initiations of treatment for each subject in all cohorts during the dose escalation stage of the study.**	Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in duration. **There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.**	Clarify 7 day period between treatment initiation of the sentinel (first) subject in a cohort and additional subjects in the cohort.
Section 3 Protocol Synopsis, Route of Administration	Taken orally in the fasting state (at least 2 hours after a meal) and at least 2 hours before another meal.	Taken orally in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal.	

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 4 Schedule of Assessments Table 2 Footnotes	25. The subject will be dispensed INXN-1001 for self-administration on **days** 2 through 7. The first INXN-1001 dose will be given at the clinical site in a fasted state (at least 2 hours after a meal, and at least 2 hours before a meal) and then the remaining 6 doses will be self-administered at the same time every day (±1 hour) in the fasted state. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day **8** visit of each cycle. Please refer to Section 8.2.5 for additional information. 30. Subjects without objective evidence of disease progression should continue to have tumor assessments performed at 8 -10 weeks intervals until **immune-related disease progression (irPD)** has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.	25. The subject will be dispensed INXN-1001 for self-administration on **Days** 2 through 7. The first INXN-1001 dose will be given at the clinical site in a **fed** state **(30 minutes after the start of a normal meal)** and at least **4** hours before another meal and then the remaining 6 doses will be self-administered at the same time every day (±1 hour) in the **fed** state. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day **7** visit of each cycle. Please refer to Section 8.2.5 for additional information. 30. Subjects without objective evidence of disease progression should continue to have tumor assessments performed at 8 -10 weeks intervals until **immune-related progression of disease (irPD)** has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.	Typographical correction of study days for INXN-1001 self-administration and drug reconciliation. Improve readability.
Section 5.3.2 Preclinical Efficacy Studies with Ad-RTS-mIL-12 Figure 3		Added axes labels: Days post tumor inoculation (x-axis) Tumor volume (mm^3) (y-axis)	Clarification of Figure.
Section 5.5 Rationale for Study Design and Dose Selection	The objectives of this Phase I clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, **there will be at least 7 days between the initiations of treatment for each subject in all cohorts during the dose escalation stage of the study**.	The objectives of this Phase I clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, **there will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.**	Clarify 7 day period between treatment initiation of the sentinel (first) subject in a cohort and additional subjects in the cohort.
Section 6.2.1 Overall Study	Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned.	Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned.	Clarify 7 day period between treatment initiation of the

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Design	Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in duration. **There will be at least 7 days between the initiations of treatment for each subject in all cohorts during the dose escalation stage of the study.** In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001 (Table 1). No intrasubject dose escalation will be allowed.	Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in duration. **There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.** In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001 (Table 1). No intrasubject dose escalation will be allowed.	sentinel (first) subject in a cohort and additional subjects in the cohort.
Section 8.2.5.1 Preparation and Administration of INXN-1001	INXN-1001 capsules will be dispensed for subject oral dosing by the site pharmacy. The site must instruct the subject to take each dose in a fasted state (i.e. at least 2 hours following any food) and to not eat any food for at least 2 hours after each dose is taken.	INXN-1001 capsules will be dispensed for subject oral dosing by the site pharmacy. The site must instruct the subject to take each dose in a **fed** state **(30 minutes after the start of a normal meal)** and to not eat any food for at least **4** hours after each dose is taken.	

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101 (formerly ADA1001)
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012 Amendment 3: 20 April 2012 Amendment 4: 13 July 2012

NOTE TO INVESTIGATORS

Amendment 4 dated 13 July 2012 will be used to conduct the study in place of any preceding version of this protocol.

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1. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Throughout Protocol	Not applicable. Subjects may receive up to 3 cycles of therapy	A few minor grammatical edits, clarifications, and other adjustments have been implemented throughout the protocol and will not be individually listed in this summary, but are indicated in the "track changes" version of the study protocol. Subjects may receive up to **6** cycles of therapy	Improve readability. Increase number of cycles of therapy that may be administered.
Title Page, Page Headings and Footers.	Amendment 3: 19 April 2012	Amendment 4: 13 July 2012	Update to current protocol version and date of amendment.
Title Page	[redacted]	[redacted]	Update Sponsor contact information.
Section 3 Protocol Synopsis; No. of Subjects	30 subjects (6 subjects per dose level cohort and up to 6 additional subjects enrolled at a single dose level at or below the MTD), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any.	**Approximately** 30 subjects (**3** subjects per dose level cohort and **approximately 15** subjects enrolled **in an expansion cohort** at a single dose level at or below the MTD), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any.	Describe the modification to the study design to include an expansion cohort.
Section 3 Study Design; Number of Centers	Multi-center (approximately 5 to 10 centers)	Multi-center (approximately **8 to 12** centers)	Revise number of potential sites.
Section 3 Protocol Synopsis; Study Design	Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in	Each subject will be treated for up to **6** treatment cycles, each of 21 days in duration. **Previously enrolled subjects received 3 cycles under former versions of this protocol.** There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts	Increase the number of treatment cycles from 3 to 6. Describe the modification to

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
	duration. There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.	during the dose escalation stage of the study. In each cycle, the subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. No intrasubject dose escalation will be allowed. **Approximately 15 additional subjects will be enrolled as an expansion cohort at a single dose level at or below the MTD.**	the study design to include an expansion cohort.
Section 3 Protocol Synopsis, Exclusion Criteria	1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. .	1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. **NOTE: For the expansion cohort ONLY, if subjects received ipilimumab, a 90-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will be made.**	Clarify washout period for subjects who have been administered immunomodulating therapies prior to enrollment on study.
Section 3 Protocol Synopsis; Dose and Schedule	INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. Note: One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001	INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. Note: One accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001. **If only 2 accessible lesions are present at screening and the single injected lesion can no longer support the injection volume at subsequent cycles, then the medical monitor should be contacted for a discussion.**	Clarify procedure for injecting lesions.
Section 3 Protocol Synopsis; Dose Escalation Plan	Not applicable.	ADDED TEXT: **e) An additional 15 subjects will be enrolled as an expansion cohort at a single dose level at or below the MTD.**	Describe the modification to the study design to include an expansion cohort.
Section 3 Protocol Synopsis; Sample Size Determination	In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety	In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety	Sentence no longer relevant.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
	evaluation has been completed~~. In the event that the dose escalation phase does not result in 6 subjects in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level.~~ Based on a review of available safety and clinical response data, the SRC may identify a single dose cohort to be expanded to a total of 12 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis. Given these considerations, a total of approximately 30 subjects may be enrolled into this study, including 6 subjects enrolled in each of the 3 planned dose level cohorts and up to 6 additional subjects enrolled at a single dose level at or below the MTD. Additional subjects may also be enrolled as replacement subjects or to explore intermediate dose level cohorts, if any.	evaluation has been completed. Based on a review of available safety and clinical response data, the SRC may identify a single dose cohort to be expanded to a total of **15** evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving 3 cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis. Given these considerations, a total of approximately **27** subjects may be enrolled into this study, including 3 subjects enrolled in each of the **4** planned dose level cohorts and approximately **15** additional subjects enrolled at a single dose level at or below the MTD. Additional subjects may also be enrolled as replacement subjects or to explore intermediate dose level cohorts, if any.	
Section 3 Protocol Synopsis; Duration of Subject Participation	Each subject's participation in this study will last approximately 5½ months, including: • 30 day screening period. • 3 cycles (9 weeks) of study treatment. • Post-treatment safety assessment visit performed 28 days after the last dose of INXN-1001. • Follow-up tumor assessment visit performed 35 ± 7 days after the Post-treatment safety assessment visit.	Each subject's participation in this study will last approximately **7** months, including: • 30 day screening period. • **6** cycles (**18** weeks) of study treatment. • Post-treatment safety assessment visit performed 28 days after the last dose of INXN-1001. • Follow-up tumor assessment visit performed 35 ± 7 days after the Post-treatment safety assessment visit.	Update number of cycles subjects may receive to 6 cycles.
Section 4 Schedule of Assessments Table 2	Cycles 1, 2 &3	Assessments were rescheduled as follows: Cycles 1 – 6: Clinical assessments, clinical laboratory, subject registration, study drug administration Cycles 1, 3, 6 only: assessments of Ab responses, cytokine profile, Ad vector shedding Cycles 1, 3, 6 only Day **15** ± 1: tumor response/pharmacodynamics including imaging studies,	Revise assessments to include subjects who may be eligible to receive up to 6 cycles of therapy.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
		digital photography, biopsy/FNA, PBMC, plasma For Expansion cohort only: Cycles 1, 3, 6 only Day **7** ± 1: tumor response/pharmacodynamics including imaging studies, digital photography, biopsy/FNA, PBMC, plasma	
Section 4 Schedule of Assessments table footnotes	15. For subjects who meet inclusion and exclusion criteria. Centralized registration of eligible subjects will be completed according to a process defined by the sponsor. Appropriate cancer staging procedures should be performed during screening, including chest and abdominal CT or MRI scans. PET scans may be accepted in place of CT or MRI scans following consultation and agreement by the ZIOPHARM Medical Monitor. For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). Disease sites are to be assessed throughout the study using the same method(s) used at screening. All subjects will have at least 2 tumor response assessments performed following the initiation of study treatment: • At the Post-treatment safety assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment. • At the Follow-up tumor assessment visit (35 ± 7 days following the Post-treatment safety assessment visit). The Investigator will evaluate response to therapy according to RECIST v1.1 guidelines.	15. For subjects who meet inclusion and exclusion criteria. Centralized registration of eligible subjects will be completed according to a process defined by the sponsor. Appropriate cancer staging procedures should be performed during screening. For the purpose of this clinical trial, the following imaging is expected at screening: **a. CT of the chest, and CT (or MRI) of the abdomen and pelvis** **b. MRI (or CT) of the brain if brain metastasis are known or suspected** **c. CT or MRI of other anatomical regions as clinically indicated** For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). Disease sites are to be assessed throughout the study using the same method(s) used at screening. **Specific image acquisition guidelines will be provided by a central imaging laboratory. Chest, abdomen, pelvis imaging is required for all follow-up imaging timepoints; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated.** All subjects will have at least 2 tumor response assessments performed following the initiation of study treatment: • At the Post-treatment safety assessment visit	Clarify text pertaining to imaging procedures as part of the study.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
		(28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment. • At the Follow-up tumor assessment visit (35 ± 7 days following the Post-treatment safety assessment visit). **For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable).**	
Section 4 Schedule of Assessments table footnotes	17. Fine needle aspiration biopsies on Day 2 should be obtained 24 hours (± 4 hours) after INXN-2001 injection, which should also be after the Day 2 INXN-1001 administration. 18. Blood sample for cellular immune response (PBMC) analyses to be collected at screening, Cycle 2 Day 15, and the Post-treatment safety assessment visit. Please refer to the laboratory manual for details regarding sample processing and shipment.	17. Fine needle aspiration biopsies on Day **7** should be obtained 24 hours (± 4 hours) after INXN-2001 injection, which should also be after the Day **7** INXN-1001 administration. 18. Blood sample for cellular immune response (PBMC) analyses to be collected at screening, **Cycle 3 Day 7**, the Post-treatment safety assessment visit, **and Follow Up Tumor Assessment Visits**. Please refer to the laboratory manual for details regarding sample processing and shipment. 22. ADDED TEXT: A blood sample for PK assessment will also be obtained Cycle 3 Day 7. 29. ADDED TEXT: Visit procedures will include: • a tumor response assessment (see footnote 16), • digital photography of all visible tumors (see footnote 17), • **PBx of injected and non-injected lesions (see footnote 18)** • **PBMC collection (see footnote 19)** • documentation of any new concomitant medications since the Post-treatment safety	Clarify the assessments occurring for Cycles 1 to 6 and those pertaining to the expansion cohort.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
		assessment visit (see footnote 8), • documentation of any anti-cancer treatments received since the previous tumor assessment (see footnote 31).	
Section 5.3.2 Nonclinical Efficacy Studies with Ad-RTS-mIL-12	Not applicable.	[redacted]	[redacted]

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 5.5 Rationale for Study Design and Dose Selection	The objectives of this Phase I/II clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, there will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.	The objectives of this Phase I/II clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, there will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. **An expansion cohort will be enrolled (a total of 15 patients) at a single dose level at or below the MTD.**	Describe the modification to the study design to include an expansion cohort.
Section 6.2 Study Design and Dose Escalation Procedure	This is a single-arm, open label, Phase I dose escalation study of intratumoral injections of INXN-2001 and oral INXN-1001 in subjects with unresectable Stage III or IV melanoma. Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to three treatment cycles, each of 21 days in duration. There will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001 (Table 1). No intrasubject dose escalation will be allowed.	This is a single-arm, open label, Phase **I/II** dose escalation study of intratumoral injections of INXN-2001 and oral INXN-1001 in subjects with unresectable Stage III or IV melanoma. Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be treated for up to **6** treatment cycles, each of 21 days in duration. **Previously enrolled subjects received 3 cycles under former versions of this protocol.** There will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001 (Table 1). No intrasubject dose escalation will be allowed. **Approximately 15 subjects will be enrolled as an expansion cohort at a single dose level at or below the MTD.**	Modify the number of cycles that subjects are eligible to receive from 3 to 6 cycles. Describe the modification to the study design to include an expansion cohort.
Section 6.2.6 Dose Escalation Procedure	Not applicable.	ADDED TEXT: **e) Approximately 15 subjects will be enrolled as an expansion cohort at a single dose level at or below the MTD.**	Describe the modification to the study design to include an expansion cohort.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
Section 6.2.7 Study Stopping Rules	For toxicity grading based on laboratory tests, an abnormality must be confirmed (when appropriate) by repeat testing. For any suspected injection reaction and/or hypersensitivity reaction, a serum tryptase level will be measured.	For toxicity grading based on laboratory tests, an abnormality must be confirmed (when appropriate) by repeat testing. ~~**For any suspected injection reaction and/or hypersensitivity reaction, a serum tryptase level will be measured.**~~	
Section 7.2 Exclusion Criteria	1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug.	1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. **NOTE: For the expansion cohort ONLY, if subjects received ipilimumab, a 90-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will be made.**	Clarify washout period for subjects who have been administered immunomodulating therapies prior to enrollment on study.
Section 8.2.5.3 Preparation and Administration of INXN-2001	INXN-2001 [redacted] will be administered as an intratumoral injection 3 hours (± 30 minutes) after oral INXN-1001 dosing on Day 1 of each cycle. INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion.	INXN-2001 [redacted] will be administered as an intratumoral injection 3 hours (± 30 minutes) after oral INXN-1001 dosing on Day 1 of each cycle. INXN-2001 should be injected into a different lesion at each cycle. If only 3 accessible lesions are present, the injections will be done in sequential rotation between 2 lesions. If only 2 accessible lesions are present, then all injections will be into a single lesion. **If only 2 accessible lesions are present at screening and the single injected lesion can no longer support the injection volume at subsequent cycles, then the medical monitor should be contacted for a discussion.**	Clarify procedure for injecting lesions.
Section 8.4.2.13 Immune Response Analyses	Blood samples will be collected from the subjects at specified visits to evaluate the potential antibody and cellular immune response to INXN-2001 (adenovirus and, RTS [redacted] components) and melanoma-associated antigens. The AdVeGFP infectivity blocking type assay will be used to detect an antibody response to the adenoviral vector.[38] Antibody response to the RTS [redacted] components may be assessed by western blot and/or enzyme-linked immunosorbent assay (ELISA). Plasma and/or serum cytokine levels (e.g., IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IFN-γ, etc.) will be assayed using multiplex and/or ELISA	Blood samples will be collected from the subjects at specified visits to evaluate the potential antibody and cellular immune response to INXN-2001 (adenovirus and, RTS [redacted] components) and melanoma-associated antigens. Antibody response to the RTS [redacted] components may be assessed by western blot and/or enzyme-linked immunosorbent assay (ELISA). Plasma and/or serum cytokine levels (eg, IL-12, IFN-γ, etc.) .) will be assayed using multiplex and/or ELISA methodology.	Describe the modification to the study design to include an expansion cohort.

Section in Amended Protocol	Original Text/Section	Revised Text/Section	Rationale for Change
	methodology.		
Section 10.1 Determination of Sample Size	In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. In the event that the dose escalation phase does not result in 6 subjects in any given cohort, additional subjects may be added such that 6 total evaluable subjects are studied at that dose level. Based on a review of available safety and clinical response data, the SRC may identify a single dose cohort to be expanded to a total of 12 evaluable subjects to better define the safety, tolerability and activity of the study treatment. Should a subject discontinue study treatment before receiving three cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis.	In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. Based on a review of available safety and clinical response data, **an additional 15 subjects may be enrolled as an expansion cohort at a single dose level at or below the MTD.** Should a subject discontinue study treatment before receiving 3 cycles for reasons other than toxicity, another subject may be added to the dose cohort to provide a total of 3 or 6 subjects for full analysis.	
Section 11.4 Duration of the Study	The study is estimated to complete enrollment within 12 months. The estimated number of months to complete study visits for the last enrolled subject is approximately 4½ months (assuming 3 treatment cycles). Therefore, the total duration of this study is expected to be approximately 1 to 2 years. Each subject's participation in this study will last approximately 5½ months, including: Not applicable	The study is estimated to complete enrollment within 12 months. The estimated number of months to complete study visits for the last enrolled subject is approximately **7** months (assuming 6 treatment cycles). Therefore, the total duration of this study is expected to be approximately **1.5 to 2.5** years. Each subject's participation in this study will last approximately **7** months, including: ADDED TEXT: **The active study period refers to the study period from informed consent through the Post Treatment Safety Assessment visit.**	Clarify the definition of the study period.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101 (formerly ADA1001)
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012 Amendment 3: 20 April 2012 Amendment 4: 13 July 2012 Amendment 5: 28 September 2012

NOTE TO INVESTIGATORS

Amendment 5 dated 28 September 2012 will be used to conduct the study in place of any preceding version of this protocol.

1. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Title Page, Page Headings and Footers	Amendment 5: 28 September 2012	Updated Protocol Amendment
Title Page	**Safety Reporting:** [redacted]	Personnel change
Section 3 Protocol Synopsis Exclusion Criteria	12. Local infection at site of injectable lesion requiring anti-infective therapy within 2 weeks of the first dose of study drug.	Additional exclusion criterion included in protocol
Section 4 Table 2 Schedule of Assessments Footnotes	Table 2 now indicates required Vital Signs "X[28]" on Day 4 of Cycle 1 and Cycle 2 for blood pressure assessment. Adenoviral vector shedding assessment now states "Cycle 1 only" Normal Skin biopsies are now indicated as **PBx or** FNA 6. Vital signs include blood pressure, pulse, temperature, and respirations. On Day 1 of each cycle, vital signs will be recorded prior to INXN-1001 dosing and hourly for the first two hours after INXN-2001 dosing. On Day 4 of Cycle 1 and Cycle 2, blood pressure is to be monitored closely with hydration as needed to prevent hypotension for up to 72 hours after administration of INXN-1001. Blood pressure assessment is required on Day 4 for Cycle 1 and Cycle 2.	Include instruction to monitor blood pressure following INXN-1001 administration. Allow for either type of biospy Include instruction to monitor blood pressure following INXN-1001 administration.

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	17. Digital photograph(s) of all visible injected tumor(s) and non-injected tumors, and of any visible local reactions in or around the injected lesion(s). Photographs are required at screening, Cycle 1 Day 7 or 15, Cycle 6 Day 7 or 15, PTSA and FUTA visits and ad hoc as the investigator deems necessary. For any subject who has or develops vitiligo, this should be documented the same way. Details regarding photographic methodology will be provided in the Photography Operations Manual. 18. Fine needle aspiration biopsies should be obtained on Day 7 ~~should be obtained 24 hours (± 4 hours) after INXN-2001 injection, which should also be~~ after ~~the~~ ~~Day 7~~ INXN-1001 administration.	Clarify photography assessment timing
	24. Intratumoral INXN-2001 [redacted] injection should be given 3 hours ± 30 minutes after the INXN-1001 dose. Lesions displaying signs of local infection should not be injected. Subjects must be adequately hydrated on each day of study drug administration. Subjects should be instructed to maintain good oral hydration on dosing days. Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions, for at least 2 hours following the INXN-2001 injection. A detailed description of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions will be documented. The subject should be instructed to call the clinical site if any such reactions develop or don't resolve within 24 to 48 hours. Please refer to Section 8.2.5 for additional information. 25. The subject will be dispensed INXN-1001 for self-administration on Days 2 through 7. Subjects must be adequately hydrated on each day of study drug administration. Subjects should be instructed to maintain good oral hydration on dosing days. The first INXN-1001	Clarify lesions displaying signs of localized infection are not to be injected. Indicate that adequate hydration is required during study drug administration.

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	dose will be given at the clinical site in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal and then the remaining 6 doses will be self-administered at the same time every day (±1 hour) in the fed state. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day 7 visit of each cycle. Please refer to Section 8.2.5 for additional information. 28. Blood pressure will be assessed at Cycle 1 and Cycle 2 Day 4 visit. For Cycles 3-6, Day 3 and Day 4 visits should be performed when feasible (e.g., Monday through Friday).	
Section 7.2 Exclusion Criteria	12. Local infection at site of injectable lesion(s) requiring anti-infective therapy within 2 weeks of the first dose of study drug.	Additional exclusion criterion included in protocol
Section 8.2.3 Treatment Regimen	Each subject's assigned INXN-1001 dose will be given orally for the first 7 consecutive days of each 21 day cycle. INXN-2001 will be given as intratumoral injections [redacted] in 0.5 mL per treatment. Following Cycle 1, if no accessible lesion for INXN-2001 injection is present (e.g., due to complete resolution), then INXN-2001 should be injected into a draining lymph node of a previously accessible lesion. The injections will be administered on the first day of each cycle throughout the study. Subjects must be adequately hydrated on each day of study drug administration. Subjects should be instructed to maintain good oral hydration in on dosing days. Blood pressure should be monitored regularly.	Indicate that adequate hydration and blood pressure monitoring are required during study drug administration.

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 8.2.5.3 Preparation and Administration of INXN-2001	The INXN-2001intratumoral administration should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or tumor-involved lymph node, at approximately 0.5 cm apart three dimensionally, to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. A detailed description and photograph of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions should be documented. Lesions displaying signs of local infection should not be injected.	Clarify lesions displaying signs of localized infection are not to be injected.
Section 8.2.5.5 Vital Signs	Vital signs will include blood pressure, pulse, temperature, and respiration. Subject's blood pressure is to be monitored closely with hydration as needed to prevent hypotension for up to 72 hours after administration of INXN-1001. Blood pressure assessment is required on Day 4 for Cycle 1 and Cycle 2.	Include instruction to monitor blood pressure following INXN-1001 administration.
Section 8.4.4 Efficacy Assessments	Appropriate cancer staging procedures should be performed during screening. ~~including chest and abdominal CT or MRI scans. PET scans may be accepted in place of CT or MRI scans following consultation and agreement by the ZIOPHARM Medical Monitor.~~ For the purpose of this clinical trial, the following imaging is expected at screening: a. CT of the chest, and CT (or MRI) of the abdomen and pelvis b. MRI (or CT) of the brain if brain metastasis are known or suspected c. CT or MRI of other anatomical regions as clinically indicated	Language updated to clarify tumor response assessment at Post-Treatment Safety Assessment visit, and to match footnote 16 in Schedule of Assessments

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines.[1] Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). For each subject, disease sites are to be assessed throughout the study using the same method(s) of assessment used at screening. Specific image acquisition guidelines will be provided by a central imaging laboratory. Chest, abdomen, pelvis imaging is required for all follow-up imaging timepoints; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated. All subjects will have at least 2 tumor response assessments performed ~~within 2 weeks prior~~ at to the Post-Treatment Safety Assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment and again at the Follow-Up Tumor Assessment visit (35 ± 7 days following the Post-treatment safety assessment visit).	
Section 11.4 Duration of the Study	Each subject's participation in this study will last approximately 7 months, including: • 30 day screening period. • 6 cycles (~~9~~18 weeks) of study treatment.	Corrected typo

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101 (formerly ADA1001)
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 21 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012 Amendment 3: 20 April 2012 Amendment 4: 13 July 2012 Amendment 5: 26 September 2012 Amendment 6: 01 March 2013

NOTE TO INVESTIGATORS

Amendment 6 dated 01 March 2013 will be used to conduct the study in place of any preceding version of this protocol.

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1. Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Title Page, Page Headings and Footers	Amendment 6: 01 March 2013	Updated Protocol Amendment
Global	Corrections in grammar, style	Clarity and accuracy
Section 3 Protocol Synopsis Exclusion Criteria	**Revised text:** 1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. **NOTE:** For the expansion cohort ONLY, if subjects received ipilimumab, a ~~90~~ 45-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will be made.	Revised wash-out period from last dose of ipilimumab from 90 days to 45 days
Section 3 Protocol Synopsis Route of Administration	**Added text:** INXN-1001: Taken orally in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal. INXN-2001: Intratumoral injection on the first day of each cycle into one accessible tumor lesion or tumor-involved (palpable) draining lymph node when necessary. Radiologically-guided injections are permitted under BL2 conditions. The injection should be administered 3 hours ± 30 minutes after the first dose of INXN-1001.	Clarify that radiographically-guided injections are permitted if available

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 3 Sample Size Determination	Given these considerations, a total of approximately ~~27~~ 30 subjects may be enrolled into this study, including 3 subjects enrolled in each of the 4 planned dose level cohorts and approximately 15 additional subjects enrolled at a single dose level at or below the MTD.	Modified for consistency in the protocol
Section 4 Table 2 Schedule of Assessments and Footnotes	**Deleted Assessments:** FOR SUBJECTS IN THE EXPANSION COHORT Removed punch biopsies and fine needle aspirate sampling from Cycle 1, Cycle 6, the Post-Treatment Safety Assessment and the Follow-Up Tumor Assessment 6. Vital signs include blood pressure, pulse, temperature, and respirations. On Day 1 of each cycle, vital signs will be recorded prior to INXN-1001 dosing and hourly for the first two hours after INXN-2001 dosing. Blood pressure is to be monitored closely ~~with hydration as needed~~ to prevent hypotension for up to 72 hours after administration of INXN-1001. Blood pressure assessment is required on Day 4 for Cycle 1 and Cycle 2. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted] 18. Punch biopsies (PBx) ~~or fine needle aspirate (FNA) samples~~ of normal skin tissue, tumor(s) and/or associated tumor involved draining lymph nodes will be collected for	Revised biopsy sampling to simplify assessments Add specific guidance regarding the need for subjects to maintain hydration with treatment

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	in vivo assessment of transgene function and immunological activities, and other biological effects should be obtained at the time points indicated in the preceding table. Details of these procedures are described in the laboratory manual. PBx: Punch biopsy (≥4 mm in diameter); ~~FNA: fine needle aspiration biopsy~~ • At screening Day -6 to -2, punch biopsies should be obtained only after subject registration has been completed (i.e., after approval by sponsor for study participation) and before start of treatment. • ~~Fine needle aspiration biopsies should be obtained on Day 7 after INXN-1001 administration.~~ • ~~Biopsies not required at Follow-up Tumor Assessment visit if ≤30 days since last biopsy.~~ 24. Intratumoral INXN-2001 (1.0 x 1012 vp) injection should be given 3 hours ± 30 minutes after the INXN-1001 dose. Lesions displaying signs of local infection should not be injected. Subjects must be adequately hydrated on each day of study drug administration, and ~~. Subjects should~~ must be instructed to maintain good oral hydration on dosing days. Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions, for at least 2 hours following the INXN-2001 injection. A detailed description of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions will be documented. The subject should be instructed to call the clinical site if any such reactions develop or don't resolve within 24 to 48 hours. Please refer to Section 8.2.5 for	

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	additional information. 25. The subject will be dispensed INXN-1001 for self-administration on Days 2 through 7. Subjects must be adequately hydrated on each day of study drug administration, and ~~. Subjects should~~ must be instructed to maintain good oral hydration on dosing days. [redacted] The first INXN-1001 dose will be given at the clinical site in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal and then the remaining 6 doses will be self-administered at the same time every day (±1 hour) in the fed state. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day 7 visit of each cycle. Please refer to Section 8.2.5 for additional information.	
Section 5.4.1 INXN-1001 and INXN-2001	[redacted]	[redacted]

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	[redacted]	[redacted]
Section 7.2 Exclusion Criteria	**Revised text:** 1. Any prior anti-cancer therapy or investigational agent within 28 days prior to the first dose of study drug. **NOTE:** For the expansion cohort ONLY, if subjects received ipilimumab, a ~~90~~ 45-day washout period since last dose of ipilimumab is required. If subjects received other immunomodulating therapies (eg, anti-PD1 antibodies), the medical monitor should be contacted and an evaluation will	Revised wash-out period from last dose of ipilimumab from 90 days to 45 days

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	be made.	
Section 8.2.3 Treatment Regimen	**Added text:** INXN-2001 will be given as intratumoral injections of approximately 1.0 x 10^{12} vp in 0.5 mL per treatment. Following Cycle 1, if no accessible lesion for INXN-2001 injection is present (eg, due to complete resolution), then INXN-2001 should be injected into a tumor-involved draining lymph node of a previously accessible lesion. The injections will be administered on the first day of each cycle throughout the study. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted] ~~ubjects must be adequately hydrated on each day of study drug administration. Subjects should be instructed to maintain good oral hydration on dosing days.~~ Blood pressure should be monitored Cycle 1 and 2 Days 1, 2, 4, and regularly thereafter.	Clarify that tumor-involved lymph nodes may be injected Add specific guidance regarding the need for subjects to maintain hydration with treatment
Section 8.2.5.3 Preparation and Administration of INXN-1001	**Added Text:** The INXN-2001 intratumoral administration should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or tumor-involved lymph node, at approximately 0.5 cm apart three dimensionally, to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the	Clarify that radiographically-guided injections are permitted if available

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	circumference of the tumor margins. Radiographically-guided injections are permitted under BL2 conditions. A detailed description and photograph of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions should be documented. Lesions displaying signs of local infection should not be injected.	
Section 8.4.2.5 Vital Signs	Vital signs will include blood pressure, pulse, temperature, and respiration. Blood pressure is to be monitored closely ~~with hydration as needed~~ to prevent hypotension for up to 72 hours after administration of INXN-1001. Blood pressure assessment is required on Day 4 for Cycle 1 and Cycle 2. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. [redacted]	Add specific guidance regarding the need for subjects to maintain hydration with treatment

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma
Protocol Number:	ATI001-101
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Original protocol: 29 April 2011 Amendment 1: 22 June 2011 Amendment 2: 13 January 2012 Amendment 3: 20 April 2012 Amendment 4: 13 July 2012 Amendment 5: 26 September 2012 Amendment 6: 01 February 2013 Amendment 7: 29 April 2013

NOTE TO INVESTIGATORS

Amendment 7 dated 29 April 2013 will be used to conduct the study in place of any preceding version of this protocol.

Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Title Page, Page Headings and Footers	Amendment 7: 29 April 2013 [redacted]	[redacted]
Abbreviations and Definitions of Terms	Several new abbreviations were included: eg, MCV, RXR	Clarify abbreviations
Global	• ~~Expansion cohort~~ is replaced by Phase II • Appendix 5 was created for the dose and schedule of subjects enrolled under the Phase I and Phase II, Group 1 portions of this study • ~~Nonreplicative~~ has been replaced by replication-incompetent for descriptions of the adenoviral vector INXN-2001 • Corrections in grammar, style	Clarity and accuracy
Section 3 Protocol Synopsis Objectives	**Revised text:** The primary objective is to: • Evaluate the safety and tolerability of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) ~~at a constant dose~~ in combination with ~~inter-cohort escalating doses of~~ INXN-1001 (activator ligand) in subjects with unresectable Stage III or IV melanoma. The secondary objectives are to: • Inform the selection of an INXN-1001 dose(s) and regimen for further study in combination with INXN-2001.	Align with current study objectives

<table>
<tr><th>Section in Amended Protocol</th><th>Revised or Deleted Text/Section</th><th>Rationale for Change</th></tr>
<tr><td>Section 3
Protocol Synopsis
No. of Subjects</td><td>Added text:

Approximately 30 subjects

(3 subjects per dose level cohort in Phase I and approximately 15 subjects enrolled in ~~an expansion cohort~~ Phase II at a single dose level at or below the MTD), exclusive of replacement subjects or the addition of intermediate dose level cohorts, if any</td><td>Update study design and clarify Phases</td></tr>
<tr><td>Section 3
Protocol Synopsis
Study Design</td><td>Added table:

Table 1: Study Design
<table><tr><th>Study Part</th><th>Cohorts</th><th>Dose Regimen</th></tr><tr><td>Phase I</td><td>Dose Escalation</td><td>INXN-2001: [redacted]
INXN-1001: 5, 20, 100, 160 mg</td></tr><tr><td>Phase II</td><td>Group 1</td><td>INXN-2001: [redacted]
INXN-1001: 160 mg daily for 7 consecutive days; 21-day cycle</td></tr><tr><td></td><td>Group 2</td><td>INXN-2001: [redacted]
INXN-1001: 160 mg every other day for 14 days; 28-day cycle</td></tr></table>Added/revised text:

In Phase I, four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned (Table 1). Subject enrollment and dose escalation will proceed according to a standard 3+3 design. ~~Each subject will be treated for up to 6 treatment cycles, each of 21 days in duration. Previously enrolled subjects received 3 cycles under former versions of this protocol. There will be at least 7 days between the initiations of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study.~~ In each cycle, the subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral ~~daily~~ doses of INXN-1001. No intrasubject dose escalation will be allowed.

[Please refer to Appendix 5 for additional study design details for the Phase I</td><td>Update study design and revise descriptions of the study Phases</td></tr>
</table>

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	portion of this study.] In Phase II, approximately 15 additional subjects will be enrolled ~~as an expansion cohort~~ at a single dose level at or below the MTD. Phase II of the study will include two groups of subjects. Subjects enrolled in the Phase II portion of the study under Amendment 6 or prior will be enrolled into Group 1; subjects enrolled under Amendment 7 or later will be enrolled into Group 2. Subjects in Group 1 will be treated with one intratumoral injection of INXN-2001 in combination with oral doses of INXN-1001 given at 160 mg daily for 7 consecutive days at the beginning of a 21-day cycle. Subjects in Group 2 will be treated with one intratumoral injection of INXN-2001 in combination with oral doses of INXN-1001 given at 160 mg every other day for 14 days at the beginning of a 28-day cycle. Subjects may receive treatment for 6 cycles.	
Section 3 Protocol Synopsis Inclusion Criteria	**3.** A minimum of 2 accessible ~~nonvisceral~~ lesions (shortest diameter ≥ 1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥ 1.5 cm).	Revise text for clarity
Section 3 Protocol Synopsis Dose and Schedule	**Deleted text:** ~~All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 3-week cycle. The subjects will also receive a single daily oral dose of INXN-1001 for 7 consecutive days starting on Day 1 of each cycle. Subjects may receive up to 6 cycles of study treatment~~. **Added text:** Phase II, Group 2: All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of a 4-week cycle. The subjects will also receive a single oral dose of INXN-1001 every other day for 14 days starting on Day 1 of each cycle. Subjects may receive up to 6 cycles of study treatment.	Update study design and revise descriptions of the study Phases
Section 3 Protocol Synopsis Dose Escalation Plan	The **Dose Escalation Plan**, **Definition of MTD, and Definition of DLT** Sections were deleted from the Synopsis and added to Appendix 5	Modified to simplify the protocol

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Definition of MTD Definition of DLT		
Section 3 Protocol Synopsis Duration of Subject Participation	**Revised text:** ~~Each~~ Phase II, Group 2 subjects' participation in this study will last approximately ~~9~~ 8 months, including: • 30 day screening period. • 6 cycles (~~18~~ 24 weeks) of study treatment. • Post-Treatment Safety Assessment visit performed 28 days after the last dose of INXN-1001. • Follow-Up Tumor Assessment visit performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. In addition, subjects who discontinue or complete study treatment in the absence of rapid clinical deterioration and without objective evidence of confirmed immune-related ~~disease~~ progressi~~on~~ve disease (irPD) should continue to be followed until ~~immune-related disease progression (~~irPD~~)~~ has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first.	Update study design, revise descriptions of the study Phases and duration of subject participation
Section 3 Protocol Synopsis **Sample Size Determination**	**Revised text:** Given these considerations, a total of approximately 30 subjects may be enrolled into this study, including 3 subjects enrolled in each of the 4 planned dose level cohorts and approximately 15 additional subjects enrolled ~~at a single dose level~~ at or below the MTD during the Phase II portion of the study. Additional subjects may also be enrolled as replacement subjects or to explore intermediate dose level cohorts~~, if any~~.	Update study design and revise descriptions of the study Phases
Section 4 Table 2 Schedule of Assessments and Footnotes	**Assessments included are for all Phase II Group II subjects; Phase I and Phase II Group I Schedule of Assessments are now located in Appendix 5** • Day 3 ~~&~~OR Day 4 • Day ~~8 OR Day 9~~13 • Day 15 ~~± 2 days~~	Clarify Study visits and Assessments; simplify schedule

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	• Oral INXN-1001 Day 1 – Day ~~7~~14 • Cytokine profile: added Day 3 • PK: deleted ~~Day 3~~ • Deleted ~~Ad Vector Shedding~~ • Deleted ~~FNA~~ • Footnote numbering has changed **Footnotes**: 6. Vital signs include blood pressure, pulse, temperature, and respirations. On Day 1 of each cycle, vital signs will be recorded prior to INXN-1001 dosing and hourly for the first two hours after INXN-2001 dosing. Blood pressure is to be monitored closely to prevent hypotension ~~for up to 72 hours~~ after administration of INXN-1001. Blood pressure assessment is required on ~~Day 3 or~~ Day 4 for Cycle 1 and Cycle 2. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. Oral hydration is of paramount importance ~~during the first 72 hours~~ after first injection of INXN-2001 because most toxicity has been observed during this time period. 7. Monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. AEs/SAEs that occur following informed consent until the Post-Treatment Safety Assessment must be recorded on the AE CRF~~; AEs/SAEs that occur prior to informed consent should be added to the medical history CRF. Study drug-related AEs/SAEs that are ongoing at the time of the Post-Treatment Safety Assessment visit should be followed until resolution, return to baseline, they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor), or they can be attributed to other than the study drug or other than study conduct. SAEs considered to be related to study drug that occur beyond 30 days after the last dose must also be reported.~~ 8. In addition, all SAEs must be reported by the investigator or designee within 24 hours of becoming aware of the event, from the time of	

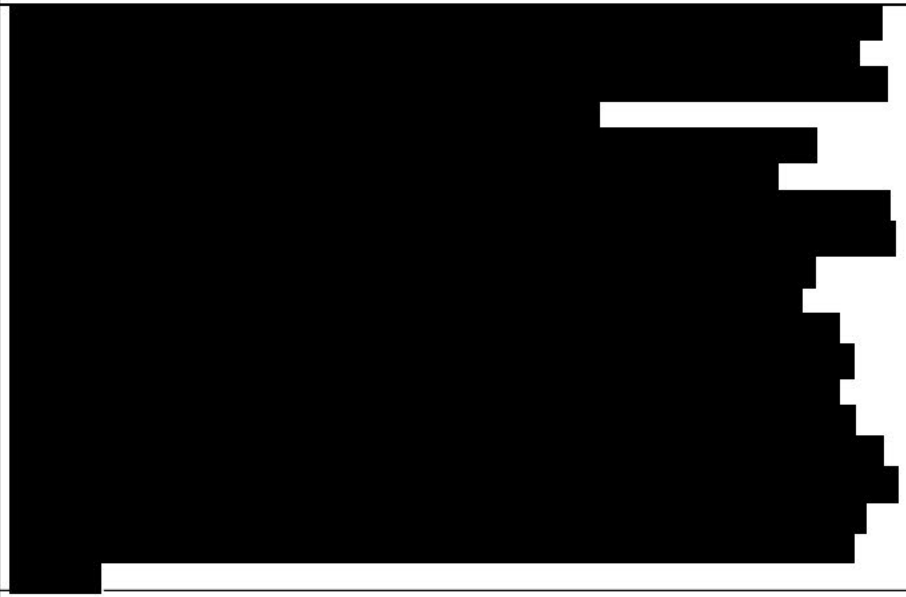
Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	informed consent through 30 days after the last dose of study drug, regardless of the initiation of any new anti-cancer therapy. ~~SAEs considered to be related to study drug that occur beyond 30 days after the last dose must also be reported~~. 10. Hematology tests include: complete blood count and white blood cell count, differential white blood cell count, red blood cell count, hematocrit, hemoglobin, red blood cell indices, reticulocyte count, MCV (mean corpuscular volume) and platelet count. PTT (partial thromboplastin time) and INR (international normalized ratio) will also be evaluated. 16. ~~Serum~~ Blood sample for analysis of antibody responses to the adenovirus and/or the RTS components, and antibodies to melanoma-associated antigens. Please refer to the laboratory manual for details regarding sample processing and shipment. 17. Blood samples for cytokine profiling. Please refer to the laboratory manual for details regarding sample processing and shipment. 18. For subjects with measurable lesions, target lesions should be selected and measured as per RECIST 1.1 guidelines. Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be measured (if measureable). Disease sites are to be assessed throughout the study using the same method(s) used at screening. Specific image acquisition guidelines will be provided by a central imaging laboratory. Chest, abdomen, pelvis imaging is required for all follow-up imaging time points; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated. ~~All subjects will have at least 2 tumor response assessments performed following the initiation of study treatment:~~ ~~At the Post-Treatment Safety Assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment.~~ ~~At the Follow-Up Tumor Assessment visit (35 ± 7 days following the~~	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~Post-Treatment Safety Assessment visit).~~ 19. Digital photograph(s) of all visible injected tumor(s) and non-injected tumors, and of any visible local reactions in or around the injected lesion(s). Photographs are required at screening, Cycle 1 Day ~~7 or 15~~13, Cycle 6 Day ~~7 or 15~~13, PTSA and FUTA visits and ad hoc as the investigator deems necessary. 20. For Phase II: blood samples for cellular immune response (PBMC) analyses are to be collected at screening, Cycle 3 Day 13, the Post-Treatment Safety Assessment, and Follow-Up Tumor Assessment visits. Please refer to the laboratory manual for details regarding sample processing and shipment. **NOTE:** If a subject is off-study before Cycle 3, then a blood sample will be obtained at the PTSA visit. 24. The first INXN-1001 dose will be given on Day 1 at the clinical site in a fed state (30 minutes after the start of a normal meal) and at least 4 hours before another meal and then the remaining 6 doses will be self-administered every other day at the same time (±1 hour) in the fed state. Subjects must be adequately hydrated on each day of study drug administration, and must be instructed to maintain good oral hydration on and between dosing days. Oral hydration is of paramount importance through Cycle 2 following injection of INXN-2001 because most toxicity has been observed during this time period. Study sites must verify compliance with INXN-1001 dosing on Days 5, 11, and 13. Subjects should return bottles of INXN-1001 for reconciliations to determine extent of subject adherence to self-administration preferably on the Day 15 visit of each cycle. Please refer to Section 8.2.5 for additional information. 26. FOR CYCLE 1 ONLY: Subjects will undergo an INXN-1001 pharmacokinetic (PK) assessment. Blood samples for INXN-1001 PK analysis should be obtained on the following days and time points: Day 1: Pre-dose (< 30 minutes prior to INXN-1001 dosing) 1-2 hours post INXN-1001 dosing 3-~~8~~ 6 hours post INXN-1001 dosing Day 2: Pre-dose (< 30 minutes prior to INXN-1001 dosing)	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Day ~~7~~13:1-2 hours post INXN-1001 dosing 3-~~8~~ 6 hours post INXN-1001 dosing ~~Day 8 OR~~ Day ~~9~~15: anytime >24 hours post-Day ~~7~~13 INXN-1001 dose Plasma samples will be obtained for determining levels of INXN-1001, IL-12 and interferon-gamma for subjects in the dose escalation cohorts on Cycle 3 Day 15, and for subjects in the expansion cohort on Cycle 3 Day 7. 27. Blood pressure will be assessed at Cycle 1 and Cycle 2 Day 3 or 4 visit. During Cycle 1 and 2, Day 3 or 4 visits are required. For Cycles 3-6, Day 3 or Day 4 visits should be performed when feasible (eg, Monday through Friday). 28. Post- Treatment Safety Assessment visit will be performed 28 ± 3 days after the last dose of INXN-1001 or at the time of early withdrawal from study treatment. ~~Note: imaging studies performed for the tumor response assessment may be completed within 2 weeks prior to the Post-Treatment Safety Assessment visit.~~ ~~Subjects with ongoing study drug-related AEs/SAEs should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor), or they can be attributed to other than the study drug or other than study conduct. Subjects found to have residual viral particles or DNA in tissue or blood samples may be requested to return to the clinic at monthly intervals for further testing until clearance has been documented.~~ 29. A Follow-Up Tumor Assessment visit will be performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. This assessment is required for all subjects, including those with prior objective evidence of disease progression to ensure that more slowly declining tumor burden in response to therapy is not missed. ~~Visit procedures will include:~~ ~~• a tumor response assessment (see footnote 16),~~ ~~• digital photography of all visible tumors (see footnote 17),~~	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~• PBx of injected and non-injected lesions (see footnote 18),~~ ~~• PBMC collection (see footnote 19),~~ ~~• documentation of any new concomitant medications since the Post-Treatment Safety Assessment visit (see footnote 8),~~ ~~• documentation of any anti-cancer treatments received since the previous tumor assessment (see footnote 31).~~ Subjects without objective evidence of disease progression should continue to have tumor assessments performed at 8 -10 weeks intervals until ~~immune-related progression of disease~~ (irPD~~)~~ has been confirmed or an alternate anti-cancer therapy has been initiated, whichever occurs first. 30. Interim cancer history information will include documentation of any new concomitant medications and any anti-cancer treatments received since the Post-Treatment Safety Assessment visit or previous tumor assessment	
Section 5.1 Disease Background	**Added text:** Other immunotherapies for the treatment of melanoma are also in various stages of development, including immune checkpoint inhibitors, and OncoVEX^{GM-CSF}, an oncolytic herpes simplex virus encoding GM-CSF.	Add new immunotherapies in development
Section 5.4.1 INXN-1001 and INXN-2001	**Added text:** Please refer to the current Investigator's Brochure for additional information regarding the previous nonclinical and clinical experience with INXN-1001 and INXN-2001. [redacted]	Update the safety profile in subjects receiving study drug

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	[redacted] **Deleted text:** [redacted]	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
		
Section 5.5 Rationale for Study Design and Dose Selection	**Revised text:** The objectives of this Phase I/II clinical trial are to assess the safety, objective response rate, and immunological and biological effects of intratumoral injections of INXN-2001 in combination with escalating dose levels of oral INXN-1001. The dose escalation portion of the study (Phase I) uses a standard 3+3 design commonly used in Phase I studies of investigational anticancer therapies. To provide an opportunity to monitor the safety of the combination therapy, there will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. ~~An expansion cohort~~ Phase II will be enrolled (~~a total of~~ approximately 15 subjects) at ~~a single dose level or~~ below the MTD and using two groups. One group will receive an INXN-1001 daily for 7 days dosing regimen, and the other group will receive an every other day dosing regimen for 14 days to compare tolerability of INXN-1001 at the 160-mg dose.	Update study design and revise descriptions of the study Phases

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Phase I: In each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. Phase II, Group 1: In each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. Phase II, Group 2: In each of six 28-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001 administered every other day for 14 days. The INXN-1001 dose levels to be studied (5 to 160 mg/day) are within the range of doses administered in previous human clinical trials [redacted] and are expected to provide plasma levels that are biologically effective for RTS activation. . ~~The 7-day duration of INXN-1001 administration is based on recent studies in mouse tumor models, which demonstrated that 7 days of INXN-1001 dosing was sufficient to induce a high level of therapeutic efficacy.~~ Although good safety and tolerability is expected based on previous clinical experience, rigorous safety monitoring during the course of this study will be performed by the Safety Review Committee (SRC).	
Section 6.1 Study Objectives	**Revised text:** The primary objective is to: • Evaluate the safety and tolerability of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) ~~at a constant dose~~ in combination with ~~inter-cohort escalating doses of~~ INXN-1001 (activator ligand) in subjects with unresectable Stage III or IV melanoma. The secondary objectives are to: Inform the selection of an INXN-1001 dose(s) and regimen for further study in combination with INXN-2001.	Align with current study objectives
Section 6.2.1 Overall Study Design	**Revised text:** Phase I: Four sequential dose escalation cohorts of INXN-1001 in combination with a fixed dose of INXN-2001 are planned. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. Each subject will be	Update study design and revise descriptions of the study Phases

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	treated for up to 6 treatment cycles, each ~~of~~ 21 days in duration. Previously enrolled subjects received 3 cycles under former versions of this protocol. There will be at least 7 days between the initiation of treatment for the sentinel (first) subject and additional subjects in all cohorts during the dose escalation stage of the study. In each cycle, subjects will be treated with one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001 ~~(Table 1)~~. No intrasubject dose escalation will be allowed. Phase II: Approximately 15 subjects will be enrolled ~~as an~~in ~~expansion cohort~~Phase II ~~at a single dose level~~ at or below the MTD. For Group 1, in each of six 21-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral daily doses of INXN-1001. For Group 2, in each of six 28-day cycles, the subjects will receive one intratumoral injection of INXN-2001 in combination with 7 oral doses of INXN-1001 administered every other day for 14 days.	
Section 6.2.2 Treatment Parameters and Duration	**Revised text**: All subjects will receive intratumoral injections of INXN-2001 [redacted] on Day 1 of ~~a 3-week~~ each cycle. The subjects will also receive ~~a single daily~~an oral daily dose of INXN-1001 for 7 consecutive days (Phase I and Phase II Group 1) or every other day for 14 days (Phase II Group 2) starting on Day 1 of each cycle. ~~The INXN-1001 dose assigned to any given subject will be determined according to the dose escalation schedule (Section 6.2.6)~~.	Update study design and revise descriptions of the study Phases; revise dosing regimen
Section 6.2.3 Safety Review Committee (SRC)	**Revised text:** A SRC comprised of the Medical Monitor, Principal Investigators and sponsor representatives, will hold periodic teleconferences to evaluate the safety and treatment status of all subjects. The SRC will review and assess the safety data at the completion of each dose cohort as described in Section 6.2.6, and at any other time as needed. The SRC has the authority to recommend dose modifications for safety concerns. ~~The following data will be reviewed at each SRC meeting to determine whether to~~	Simplify language and clarify the role of the SRC in Phase II Group 2

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~proceed to a subsequent cohort: physical examination and vital signs; medical history; complete blood count with differential; serum chemistry panel including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin; lactate dehydrogenase (LDH); albumin; serum tryptase level; ECG; cytokine levels; and adverse event and concomitant medication records. Additional information will be reviewed as necessary.~~ A written summary documenting the results and recommendations of each review will be provided to the Investigator(s) and maintained on file with the sponsor. Additional sub-Investigators and personnel may participate in reviews as indicated. In Phase I, ~~F~~following a cohort review, the SRC may recommend proceeding with enrollment in the next dose cohort, enrolling additional subjects in the current cohort, dropping back to a lower cohort, exploring an alternate dose level, or not enrolling any additional subjects. The dose escalation and enrollment guidelines outlined in Section 6.2.6, as well as the study stopping rules outlined in Section 6.2.7, will be used as the basis for these assessments. In Phase II, Group 2, the DLT definitions in Section 6.2.4 will be utilized along with the criteria for de-escalation. The SRC may recommend a dose reduction for subjects in Group 2 if one or more DLT(s) are observed.	
Section 6.2.4 Definition and Management of Dose-limiting Toxicity (DLT)	**Added text:** Phase I and Phase II, Group 1: If DLTs have been observed in >1 of the 3-6 subjects in a cohort during the first treatment cycle, the MTD will have been exceeded and further use of that dose will be discontinued in all subjects, and the next lower dose cohort will be assessed as to whether it meets the definition of the MTD. Phase II, Group 2: If an adverse event that meets the Phase I DLT definition is observed in >1 of the 3-6 subjects in Group 2 during the first treatment cycle, the SRC will convene to discuss reduction in dose and/or change to posology and the cohort will be expanded by 3 subjects. The recommended Phase II dose will be the dosage and posology where ≤1 of 3 to 6 subjects experiences an adverse event as described in the definition of DLT.	Update study design and revise descriptions of DLT for Phase II Group 2
Section 6.2.5 Definition of Maximum Tolerated	**Revised text:** The MTD will be defined as the highest dose of INXN-1001 studied in combination with INXN-2001 at which a DLT has been seen in a maximum of ~~less~~	Update study design and revise descriptions of MTD for Phase II Group 2

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Dose (MTD) and Recommended Phase II Dose (RP2D)	~~than 2 out~~1 ~~of~~ in 6 subjects (< 33%) during the first treatment cycle. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered. The recommended Phase II dose will be the dosage and posology where ≤1 of 3 to 6 subjects experiences an adverse event as described in the definition of DLT.	
Section 6.2.6 Dose Escalation Procedure	**Revised text:** In Phase I, ~~F~~four sequential INXN-1001 dose escalation cohorts are planned, as described in ~~Table 1~~Appendix 5. Subject enrollment and dose escalation will proceed according to a standard 3+3 design. No intrasubject dose escalation will be allowed. An intermediate dose level(s) of INXN-1001 may be explored after review of all available safety and activity data, as may be decided by the SRC. Approximately 15 subjects will be enrolled in Phase II at or below the MTD. In order to increase the ability to estimate the effect of the treatment on clinical response and to correlate with immunologic or other biologic markers, an additional number of subjects may be entered into the study after the initial safety evaluation has been completed. **Moved text:** The remaining text from this Section was moved to Appendix 5.	Update study design and revise descriptions of the study Phases
Section 6.2.7 Study Stopping Rules	**Revised text:** Rules for stopping dose escalation due to DLTs observed during the first cycle of study treatment are outlined in Section 6.2.4. In the event that a given dose ~~level cohort~~ is determined to have exceeded the MTD, then further use of that dose will be discontinued for all subjects as noted in Section 6.2.4. Subsequent to Cycle 1, study drug-related adverse events that meet the DLT criteria will also be considered by the SRC and any previous determination of the MTD may be reconsidered. Stopping rules for toxicities that occur from Day 1, Cycle 2 through the Post-	Clarify study stopping rules that apply in Phase II

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	treatment follow-up visit will apply as follows: • If an adverse event can be definitely shown to be unrelated to study treatment (eg, motor vehicle accident, bee sting, elective surgery), that specific toxicity will not be considered in a stop vs. dose ~~escalation~~ modification decision. • Recruitment in the current ~~cohort~~ dose level will not be stopped for Grade 2 (NCI CTCAE v.4.03) non-dose limiting toxicities, irrespective of the number of events or number of subjects. • Recruitment in the current ~~cohort~~ dose level will be stopped for any Grade ~~3 or~~ 4 toxicity~~, unless the adverse event is clearly and incontrovertibly due to extraneous causes~~. Upon occurrence, a~~A~~ll data will be reviewed by the Investigator, the Investigator's IRB as applicable, ZIOPHARM Medical and Regulatory Staff, and the SRC to determine if the study should proceed and if/what changes must be implemented. ○ For toxicity based on laboratory tests: an abnormality related to study drug per investigator assessment must be confirmed (when appropriate) by repeat testing ~~and related to study drug per investigator~~ before qualifiying for review as above. • ~~In addition to stopping rules for dose escalation, s~~Subject enrollment into the study will be halted in the event of any study drug-related death. In this case, subject enrollment will only resume following review and approval of the SRC. ~~For toxicity grading based on laboratory tests, an abnormality must be confirmed (when appropriate) by repeat testing.~~	
Section 7.1 Inclusion Criteria	**Deleted text:** 3. A minimum of 2 accessible ~~nonvisceral~~ lesions (shortest diameter ≥1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥1.5 cm).	Clarify/revise inclusion criteria

<table>
<tr><th>Section in Amended Protocol</th><th>Revised or Deleted Text/Section</th><th>Rationale for Change</th></tr>
<tr><td>Section 8.2.3
Treatment Regimen</td><td>Added text:

Each subject's assigned INXN-1001 dose will be given orally daily for the first 7 consecutive days of each 21 day cycle (Phase I and Phase II Group 1). For Phase II Group 2 the INXN-1001 dose will be given orally every other day for 14 days of each 28 day cycle.

Oral hydration is of paramount importance ~~during the first 72 hours after~~through Cycle 2 following injection of INXN-2001 [redacted] Blood pressure should be monitored Cycle 1 and 2 Days 1, 2, 3 or 4, and regularly thereafter.</td><td>Update study design and revise descriptions of the study Phases; revise dosing regimen</td></tr>
<tr><td>Section 8.2.5
Preparation and Administration of Study Drug</td><td>Deleted text:

A combination of two investigational medications will be evaluated for safety, tolerability, efficacy, and biological effects in this trial. The small molecule INXN-1001 will be administered as oral capsules to subjects ~~once daily, for seven consecutive days,~~ in combination with an intratumoral injection of INXN-2001 (on Day 1 of each cycle). For each treatment cycle, INXN-2001 dosing will occur 3 hours ± 30 minutes after the first dose of INXN-1001.</td><td>Revise to align with dosing regimen</td></tr>
<tr><td>Section 8.2.5.2
Monitoring of Subject Adherence and Managing Missed INXN-1001 Doses</td><td>Subjects should be instructed ~~not to make up for missed doses.~~ to make up for a missed dose by taking the dose the following day, and then continuing with dosing as scheduled (eg, if the dose on Day 3 is missed, dosing should occur on Day 4 and then continue as scheduled on Days 5, 7, 9 etc). ~~Capsules that for any reason were not taken during a prior day should **not** be taken but left in the container.~~</td><td>Clarify instructions for how to continue after missing a dose with every other day dosing</td></tr>
<tr><td>Section 8.2.7
Severity Grading and Management of Injection Site Reactions</td><td>Added text:

Table 3. Injection Site Reaction Severity Grading and Management
<table>
<tr><th>CTCAE Grade</th><th>Symptoms</th><th>Course of Action</th></tr>
<tr><td>1</td><td>Tenderness with or without associated</td><td>No intervention required.</td></tr>
</table></td><td>Update language for clarification</td></tr>
</table>

<table>
<tr><th>Section in Amended Protocol</th><th>Revised or Deleted Text/Section</th><th>Rationale for Change</th></tr>
<tr><td></td><td>
<table>
<tr><td></td><td>symptoms (eg, warmth, erythema, itching)</td><td></td></tr>
<tr><td>2</td><td>Pain; lipodystrophy; edema; phlebitis</td><td>Proceed with further dosing cycles.</td></tr>
<tr><td>3</td><td>Ulceration or necrosis; severe tissue damage; operative intervention indicated</td><td>Discontinue further study treatment and notify Medical Monitor. Report and manage as DLT: see Section 6.2.4.
If toxicity meets seriousness criteria, immediately report as per SAE reporting procedures; see Section 9.4.</td></tr>
<tr><td>4</td><td>Life-threatening consequences; urgent intervention indicated</td><td>Permanently discontinue study treatment and notify Medical Monitor. Report and manage as DLT: see Section 6.2.4.
Defaults to “serious”. Immediately report as per SAE reporting procedures; see Section 9.4.</td></tr>
<tr><td>5</td><td>Death</td><td>Immediately notify Medical Monitor and report as per SAE reporting procedures; see Section 9.4.
Discontinue further subject enrollment as per study stopping rules; see Section 6.2.7.</td></tr>
</table>
</td><td></td></tr>
<tr><td>Section 8.2.8
Prophylactic Hydration, Antipyretic and/or Analgesic Administration</td><td colspan="2">The use of antipyretics and/or analgesics and aggressive hydration is allowed anytime during study treatment, as indicated, including prophylactic administration.
Since fever and flu like symptoms are commonly experienced following adenoviral vector administration it is strongly recommended that subjects be treated with prophylactic oral hydration, antipyretic and/or analgesic medications prior to study drug administration.</td><td>Add prophylactic hydration along with prophylactic antipyretics and/or analgesics</td></tr>
</table>

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	For those subjects who experience treatment related symptoms (eg, fever, headache, chills, dehydration, etc) during Cycle 1, prophylactic hydration, antipyretic and/or analgesic medications must be given prior to study drug administration in subsequent cycles. Please refer to Appendix 3 for a recommended regimen for the prophylactic administration of antipyretics and/or analgesics.	
Section 8.4.2.6 Adverse Events	**Deleted text:** . ~~Study drug-related AEs/SAEs that are ongoing at the time of the Post-Treatment Safety Assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized (and following consultation and agreement by the ZIOPHARM Medical Monitor).~~ ~~In addition, all SAEs must be reported by the investigator or designee within 24 hours of becoming aware of the event, from the time of informed consent through 30 days after the last dose of study drug, regardless of the initiation of any new anti-cancer therapy. SAEs considered to be related to study drug that occur beyond 30 days after the last dose must also be reported.~~	Deleted redundant text
Section 8.4.2.8 Hematology	**Added text:** Hematology tests include: complete blood count and white blood cell count, differential white blood cell count, red blood cell count, hematocrit, hemoglobin, red blood cell indices, reticulocyte count, MCV (mean corpuscular volume) and platelet count. PTT (partial thromboplastin time) and INR (international normalized ratio) will also be evaluated.	Clarify red blood cell indices
Section 8.4.2.14 Adenovirus Vector Shedding	**Deleted text:** ~~Subjects will be asked to provide samples of body fluids to assess any potential adenoviral vector shedding. Samples collected may include any or all of the following: whole blood, saliva and semen. Subjects will sign a consent form if they agree, but may freely decline to participate in all or any portion of this sampling without effect on their core study participation or prejudice to their future~~	Delete adenoviral shedding as an assessment

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~care.~~	
Section 8.4.3 INXN-1001 Pharmacokinetic Assessment	**Revised text:** Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day ~~8 or 9~~ 15 as defined in the Schedule of Assessments (see Table 2).	Align PK with new dosing regimen
Section 8.4.4 Efficacy Assessments	**Revised text:** All subjects will have ~~at least 2~~ tumor response assessments performed including Cycle 3 Day 13, the Post-Treatment Safety Assessment visit (28 ± 3 days following the last dose of INXN-1001) or at the time of early withdrawal from study treatment and again at the Follow-U~~up T~~tumor A~~a~~ssessment~~up tumor assessment~~ visit (35 ± 7 days following the Post-Treatment Safety Assessment visit).	Align efficacy assessments with revised study design
Section 8.4.5.1 Transgene Function	**Deleted text:** ~~FNAs of the tumor(s) and/or associated tumor involved draining lymph nodes will be collected for genomic analysis and IHC.~~	FNAs are no longer being collected as part of the study
Section 8.4.5.3 Other Biological Effects	**Deleted text:** Tumor biopsies ~~and FNAs~~ will also be assessed for changes in the frequency of apoptotic tumor cells and for other biologic effects of the treatment, such as change in tumor vasculature as a result of induction of IL-12 and other cytokines in the tumor microenvironment.	FNAs are no longer being collected in the study
Section 9.2.3 Causality Assessments	**Deleted text:** The Investigator will use medical consideration to determine the potential relationship of the AE to the study drugs based on his/her clinical judgment. ~~For SAEs, the Sponsor will provide a separate assessment of causality. The Investigator's assessment and/or the Sponsor's assessment will be considered for expedited reporting of SAEs per relevant regulatory requirements.~~	Revise safety language for clarity

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Section 9.4 Reporting Serious Adverse Events	**Revised text:** SAEs must be reported to the Sponsor or Sponsor's designee ~~immediately~~ within 24 hours of becoming aware of the event (regardless of the initiation of any new anti-cancer therapy) including the following: • Any death or SAE experienced by the patient from the signing of informed consent to 30 days after the last dose of study drug, regardless of relationship to study drug. • Any death or SAE that the Investigator becomes aware of, and believes to be study drug related, that occurs more than 30 days after the patient last received study drug. Study drug-related AEs/SAEs that are ongoing at the time of the Post-Treatment Safety Assessment visit should continue to be followed until resolution, return to baseline, or until they have stabilized or become chronic (and following consultation and agreement by the ZIOPHARM Medical Monitor). In addition to the above information, the Investigator must provide, for each event term, an assessment of ~~the following~~: • Severity ~~of the SAE~~/intensity • Relationship to the study drug (causality assessment)	Revise safety language for clarity
Section 10.3 Statistical Methods	**Added text:** Data will be summarized by dose cohort based on the actual dose of INXN-1001 received on Day 1, Cycle 1. For Phase II, data will be summarized based on group and dose regimen.	Update statistical considerations for revised study design
Section 11.4 Duration of the Study	**Revised text:** The study is estimated to complete enrollment ~~within 12~~ over 24 months. The estimated number of months to complete study visits for the last enrolled subject is approximately ~~7~~8 months (assuming 6 treatment cycles). Therefore, the total duration of this study is expected to be approximately 1.5 to 2.5 years. Each subject's participation in this study will last approximately ~~7~~8 months	Update to align with dosing regimen and cycle length

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change

Section 17
Appendix 3
	Added/revised text:
	
	Recombinant adenoviral vectors have the potential to elicit potent cellular and humoral immune responses in recipients. While the mechanism responsible for these effects is poorly understood, dehydration and transient low-grade fevers are common after systemic recombinant adenovirus vector administration and temperatures up to 104° F with chills and generalized malaise have been observed in first and/or subsequent treatment cycles.
	Dehydration may occur in the absence of adequate hydration prophylaxis. Subjects must be instructed to maintain adequate oral hydration on and in between dosing days; sites must closely monitor subjects' hydration status. Oral hydration is of paramount importance after first injection of INXN-2001 through Cycle 2 [redacted]
	Because low grade fever is very likely to occur, prophylaxis with a non-steroidal anti-inflammatory agent (ibuprofen) or acetaminophen (if a subject cannot tolerate ibuprofen) is strongly recommended starting with Cycle 1.
	• Ibuprofen is available without a prescription in 200 mg tablets. Usually ~~1 2 tablets~~ 800 mg every 4-6-8 hours will prevent and/or decrease fever. The lowest expected efficacious dose should be used.
	
	  • Side effects of ibuprofen include nausea and vomiting, which may be prevented if the medication is taken with food. Rare side effects include diarrhea, constipation, heartburn, and stomach pain. People with stomach ulcers or kidney disease, and those with an aspirin allergy should avoid ibuprofen.
	
	  • Common brand names of ibuprofen include Advil®, Motrin®, and Nuprin®.
	
	  • Aspirin should be avoided as it may be toxic in large doses in adults.
	
	• While meta-analyses suggest that ibuprofen is a better anti-pyretic medication than acetaminophen, acetaminophen also prevents and or reduces a fever. It is available without a prescription in 325 mg or 500 mg tablets. Again, ~~1 2 tablets~~ 1000 mg every ~~4~~ 6-8 hours should be used to eliminate fever. The
		Update with language about hydration and its importance during treatment
		
		Update doses of antipyretics to be used

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	maximum dose of acetaminophen in adults should not exceed 4 grams in a 24 hour period. **Deleted Text:** ~~If you choose not to institute prophylactic anti-pyretic therapy on Cycle1, Day 1 then a subject should be instructed in how to treat fever if it occurs following viral vector injection.~~	
Section 19 Appendix 5	**Added text:** Sections of protocol synopsis referring to the Phase I and Phase II, Group 1 have been moved to Appendix 5.	Simplify the protocol by moving information about completed Phase/groups to the Appendix