STUDY PROTOCOL

Protocol Title:	A Phase II, Randomized, Open-Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions
Protocol Number:	ATI001-201
Phase:	II
Date of Protocol:	Amendment 2: 17 Jun 2013 Amendment 1: 04 Jan 2013 Original: 17 Aug 2012
Sponsor:	ZIOPHARM Oncology, Inc. [redacted]
Medical Monitor:	[redacted]
Safety Reporting:	[redacted]

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2. ABBREVIATIONS AND DEFINITIONS OF TERMS

Ad	Adenovirus
AE	Adverse Event
ALC	Absolute lymphocyte Count
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
ANC	Absolute neutrophil count
AST	Aspartate Transaminase
BSA	Body Surface Area
BUN	Blood urea nitrogen
CBC	Complete Blood Count
CBR	Clinical Benefit Rate
GCP	Good Clinical Practice
CI	Confidence interval
CR	Complete response
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLs	Cytotoxic T Lymphocytes
CYP450	Cytochrome P450
DCs	Dendritic Cells
DLT	Dose-Limiting Toxicity
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EcR	Ecdysone Receptor
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
FDA	Food & Drug Administration
FNA	Fine Needle Aspirate
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HER2	Human epidermal growth factor receptor 2
HIPAA	Health Insurance Portability and Accountability Act of 1996
hIL-12	Human Interleukin 12
IEC	Independent Ethics Committee
IFOS	ifosfamide
IHC	immunohistochemistry
IL-2	Interleukin 2
INR	International normalized ratio
INXN-1001	Small molecule Activator Ligand
INXN-2001	Ad-RTS-hIL-12
IRB	Institutional Review Board
IP	Intraperitoneal(ly)
IT	Intratumoral(ly)
IV	Intravenous(ly)
IVIG	Intravenous immunoglobulin
LDH	Lactate Dehydrogenase
MDRD	Modified Diet in Renal Disease
MDSC	Myeloid Derived Suppressor Cell
MedDRA	Medical Dictionary for Regulatory Activities
mRECIST	Modified Response Evaluation Criteria in Solid Tumors v1.1
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NK	Natural Killer
NYHA	New York Heart Association
ORR	Objective response rate
Palifosfamide	Isophosphoramide mustard, tris-salt
PBMC	Peripheral Blood Mononuclear Cells
PBx	Punch Biopsy
PD	Progressive Disease
PFS	Progression-free Survival
PI	Principal Investigator
PK	Pharmacokinetic(s)
PR	Partial response
PTT	Partial Thromboplastin Time
RBC	Red blood cell
[redacted]	[redacted]
SAE	Serious Adverse Event
SD	Stable disease
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
Tregs	Regulatory T Cell
ULN	Upper Limit of Normal
vp	Viral particles
WBC	White blood cell

2. PROTOCOL SYNOPSIS

Title	A Phase II, Randomized, Open-Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions
Protocol Number	ATI001-201
Study Sponsor	ZIOPHARM Oncology, Inc.
Clinical Phase	II
Objectives	*Primary Objectives*: • Assess the safety and tolerability of INXN-1001 (activator ligand) + INXN-2001 (Ad-RTS-hIL-12]) as monotherapy and in combination with palifosfamide in subjects with recurrent and/or metastatic adenocarcinoma of the breast with accessible lesions • Assess the efficacy of repeated cycles of intratumoral (IT) injections of INXN-2001 (Ad-RTS-hIL-12) with INXN-1001 (oral activator ligand) as monotherapy or in combination with palifosfamide as measured by the 16-week progression-free survival (PFS) rate *Secondary Objectives:* • Estimate PFS by modified Response Evaluation Criteria in Solid Tumors (mRECIST) v1.1 • Assess objective response rate (ORR) by mRECIST v1.1 • Assess duration of response • Assess clinical benefit rate • Evaluate pharmacodynamic tumor markers (eg, breast cancer-associated biomarkers such as MUC-1) • Assess the pharmacokinetics (PK) of INXN-1001
Study Drugs	INXN-1001 (oral activator ligand) INXN-2001 (Ad-RTS-hIL-12) Palifosfamide
No. Centers	Multicenter (approximately 30 centers in the US)
Anticipated No. of Subjects	Approximately 9 subjects, up to 62 subjects • Part 1 Safety Run-in: approximately 9 subjects • Part 2 Randomization: up to 53 subjects
Study Design	This is a multicenter, open-label, randomized, Phase II study to evaluate the safety and efficacy of INXN-1001 + INXN-2001 (to be

referred to as "**A**d-RTS-hIL-12 monotherapy" or Arm **A**) and INXN-1001 + INXN-2001 with palifosfamide (to be referred to as "**C**ombination therapy" or Arm **C**) in subjects with recurrent/metastatic breast cancer with accessible tumor(s). (Table 1 and Figure 1)

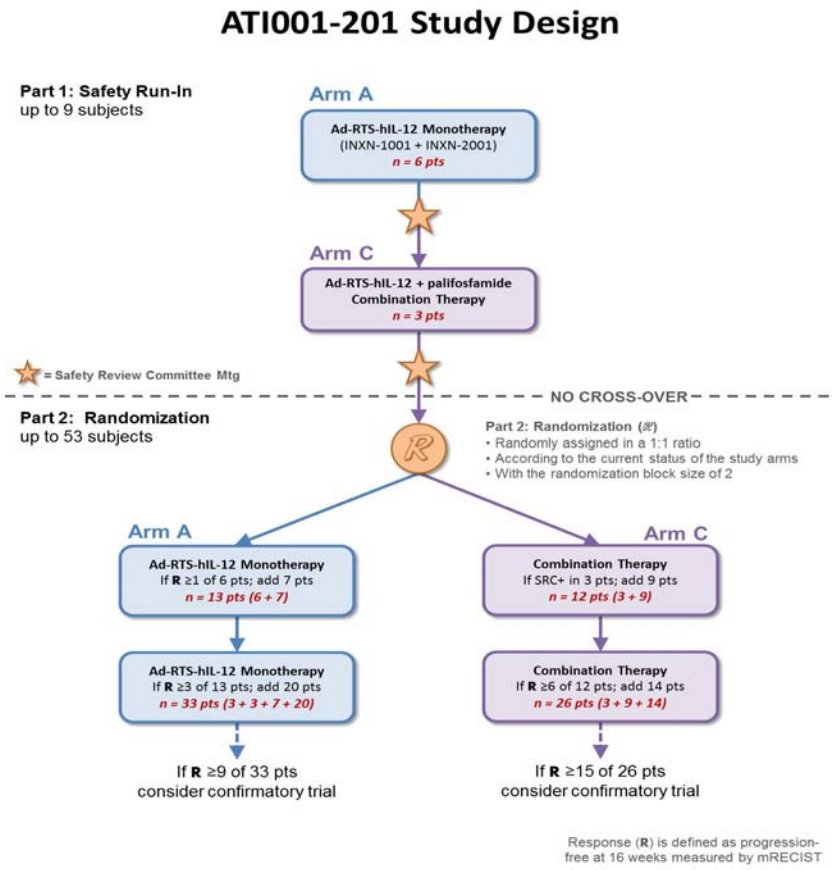
Part 1 is a safety run-in where a safety assessment will be made after 1 cycle of therapy.

In **Part 2**, eligible subjects will be randomly assigned to active treatment Arms A or C. Various futility analyses based on PFS at week 16 have been incorporated in both arms as described below.

Table 1: Study Design

Part	Treatment Arm	Intervention (21-day cycle)
1 Safety Run-in	A	Ad-RTS-hIL-12 monotherapy; n=6 [INXN-1001 (140 mg daily)+ INXN-2001 ([redacted])
	C	Combination therapy; n=3 [INXN-1001 (140 mg daily) + INXN-2001 [redacted] + palifosfamide (120 mg/m^2)]
2 Randomization	A	Ad-RTS-hIL-12 monotherapy [INXN-1001 (140 mg daily) + INXN-2001 [redacted] 10^{12} vp)]
	C	Combination therapy [INXN-1001 (140 mg daily) + INXN-2001 [redacted] + palifosfamide (120 mg/m^2)]

Figure 1: Study Design Schema



Treatment Arm A: Ad-RTS-hIL-12 Monotherapy

During **Part 1,** Arm A will explore the safety of Ad-RTS-hIL-12 monotherapy at 140 mg INXN-1001 daily for 7 days (n=6). If ≤1 of the first 6 subjects survive progression-free at week 16, then enrollment in Arm A will be stopped. If at least one of the 6 subjects survives progression-free at week 16, then Arm A will be expanded with 7 additional subjects to be randomly assigned to Arm A. These first 13 subjects (6 + 7) on Arm A will undergo tumor assessment for progressive disease (PD) after 16 weeks of treatment. If ≥3 of these 13 subjects survive progression-free at week 16, this stage will be expanded to include 20 additional randomly assigned subjects. If ≥9 of these 33 (13 + 20) subjects survive progression-free at week 16, then this regimen would be considered worthy of further investigation (ie, a confirmatory trial).

Treatment Arm C: Combination Therapy

Once the monotherapy (Arm A) is determined to be safe and tolerable, Part 1 combination therapy (Arm C) begins.

Part 1 of Arm C will evaluate the safety and tolerability of the combination of Ad-RTS-hIL-12 with palifosfamide in 3 subjects with a 1-week interval between dosing of subjects. If combination therapy is determined to be tolerable in 3 subjects following one cycle of

	treatment, then this treatment arm will be included in Part 2 of the study. During **Part 2,** 9 additional subjects will be randomly assigned to Arm C. If <6 of the first 12 (3+9) subjects survive progression-free at week 16, no additional subjects will be enrolled. If ≥6 of the 12 subjects survive progression-free at week 16, an additional 14 subjects will be randomly assigned. If ≥15 of the 26 (3 + 9 +14) subjects in the expansion cohort survive progression-free at week 16, then this regimen would be considered worthy of further investigation (ie, a confirmatory trial). The primary efficacy endpoint is the 16-week PFS rate, defined as the proportion of subjects that remain progression-free at week 16 by mRECIST v1.1 for Arms A and C. Futility decisions will be made using results at week 16 from both primary and secondary endpoints. The safety of study treatments will be assessed throughout the study by the frequency and severity of adverse events (AEs) as determined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03. An SRC meeting can be convened if deemed necessary by the sponsor or the investigators at any point during the study.
Study Population	**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. Histologically or cytologically confirmed adenocarcinoma of the breast, either locally recurrent or metastatic disease with injectable lesions, for which no proven curative therapy exists. Locally recurrent disease must not be amenable to surgical resection or radiation with curative intent. 3. Subject has failed or progressed on at least 1 prior systemic chemotherapy regimen ± biologic/experimental therapy (if first-line therapy, failure or progression during the first 30 days). 4. Resolution of all treatment-related toxicities to Grade 1 severity or lower, except for stable sensory neuropathy ≤ Grade 2 and alopecia. 5. A minimum of 2 lesion(s) assessed by imaging using mRECIST v1.1. 1 or more lesions NOT to be injected AND at least 1 injectable lesion(s) (may use radiographically-guided injection of lesions if available at site) 6. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, 2 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. Women of childbearing potential (perimenopausal women must be amenorrheic for at least 12 months to be considered of

	non-childbearing potential) must have a negative pregnancy test at screening. 8. Adequate bone marrow reserve as indicated by: a) Absolute neutrophil count (ANC) > 1500/μL (without use of growth factors within 7 days) b) Absolute lymphocyte count (ALC) > 700/μL (without use of growth factors within 7 days) c) Platelet count > 100,000/mm^3 (without transfusion in prior 7 days) d) Hemoglobin > 9.0 g/dL (without transfusion in prior 7 days) 9. Adequate renal function as evidenced by estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) equation: eGFR ≥ 60 mL/min/1.73 m^2 10. Adequate liver function as evidenced by the following: a) Bilirubin ≤ 1.5 times the upper limits of normal (ULN) b) Alanine aminotransferase (ALT), and aspartate aminotransferase (AST) ≤ 2.5×ULN, in the case of liver metastases ≤ 5×ULN **Exclusion Criteria:** 1. Subjects with human epidermal growth factor receptor 2 (*HER2)/neu*-positive (immunohistochemistry [IHC]) 3+ or fluorescence in situ hybridization-amplified) breast tumors who are eligible for, but who have not received HER2-targeted therapy (eg, trastuzumab) 2. Concomitant anticancer therapies (eg, endocrine therapy for breast cancer) 3. Prior therapies discontinuation periods: a) Radiation within 3 weeks of enrollment b) Chemotherapy within 4 weeks of enrollment c) Nitrosoureas within 6 weeks of enrollment d) Biologic therapy and/or immunomodulatory therapy (eg, granulocyte colony stimulating factor [GCSF]/granulocyte macrophage colony stimulating factor [GM-CSF], interferons or interleukins, growth hormone, intravenous immunoglobulin [IVIG], retinoic acid), checkpoint inhibitors (eg, ipilimumab, anti-PD-1 or anti-PDL-1 antibodies) within 6 weeks of enrollment e) No washout period is required for endocrine therapy 4. Radiation therapy encompassing >25% of bone marrow 5. History of bone marrow or stem cell transplantation 6. Any congenital or acquired condition leading to inability to

	generate an immune response, including concomitant immune suppressive therapy 7. Immunosuppressive therapy: a) Systemic immunosuppressive drugs including corticosteroids (prednisone equivalent >10 mg/day, http://www.medcalc.com/steroid.html) within 6 weeks b) Immune suppression/requiring immunosuppressive drugs, including subjects with organ allografts c) Active autoimmune disease requiring the equivalent of >10 mg/day of prednisone 8. Major surgery within 4 weeks of study treatment, or major surgery planned for duration of study participation 9. History of prior malignancy, unless the prior malignancy was diagnosed and definitively treated ≥5 years previously with no subsequent evidence of recurrence NOTE: This does not apply to previous breast cancer, carcinoma in situ of the cervix, inactive melanoma or non-melanoma skin cancer, or Grade 1 papillary bladder cancer 10. Subjects with brain or subdural metastases, unless local therapy has completed and corticosteroids have been discontinued for this indication for ≥4 weeks before starting study treatment. Any signs (eg, radiologic) and/or symptoms of brain metastases must be stable for ≥4 weeks before starting study treatment; radiographic stability should be determined by comparing a contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) brain scan performed during screening to a prior scan performed at least 4 weeks earlier. 11. Any medications that induce, inhibit, or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of study drug 12. Subjects with meningeal carcinomatosis 13. Known significant hypersensitivity to study drugs or excipients 14. History of malabsorption syndrome or other condition that would interfere with enteral absorption 15. 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. 16. New York Heart Association (NYHA) Class II or greater congestive heart failure OR active ventricular arrhythmia requiring medication

	17. Any other unstable or clinically significant concurrent medical condition (eg, infection requiring systemic anti-infective agents) that would, in the opinion of the investigator, jeopardize the safety of a subject and/or their compliance with the protocol 18. Localized infection at site of injectable lesion(s) requiring anti-infective therapy within 2 weeks of the first dose of study drug. NOTE: Appropriate confirmatory testing (eg, punch biopsy [PBx] with bacterial counts) must be performed to rule out the presence of infection if ambiguous, or clinical signs of infection are evident
Experimental Therapy	INXN-1001 will be supplied by the Sponsor as a capsule for oral administration. INXN-2001 will be supplied by the Sponsor as a sterile suspension for injection. Palifosfamide will be supplied by the Sponsor as a powder in single-use glass vials. **Dose & Schedule (21-day cycle):** • **INXN-1001:** Subjects will be directed to take the INXN-1001 oral dose in a fed state (within approximately 30 minutes following a normal meal), once daily at the same time each day (±1 hour), beginning on Day 1 of each cycle. Each subject will receive INXN-1001 (140 mg daily for 7 days). • **INXN-2001**: INXN-2001 [redacted] IT injection administered 3 hours (± 30 minutes) after INXN-1001 dosing on Day 1 of each cycle. If >1 injectable lesion is present, INXN-2001 will be injected into a different lesion at each cycle, and if the number of lesions is limited, the injections will be administered in sequential rotation or at investigator's discretion. If only a single lesion is present, then repeat administrations into the same lesion are permitted, and radiologically-guided techniques may be used as available. • **Palifosfamide:** Palifosfamide (120 mg/m^2/day) will be administered by intravenous (IV) infusion over approximately 30 minutes on Days 1-3 of each cycle.
Duration of Treatment	In the absence of meeting treatment withdrawal criteria, subjects should receive 6 cycles of study treatment.
Duration of Study	The duration of this study from the time of initiating subject enrollment until the completion of survival follow-up is anticipated to be approximately 34 months.
Safety Evaluations	Safety parameters will include serious adverse events (SAEs), AEs, physical examinations, electrocardiograms (ECGs), vital signs, clinical laboratory evaluations, medical history, and prior/concomitant medications. A safety review for each arm of the study will occur following 1 cycle of therapy during Part 1.

Efficacy Evaluations	The primary efficacy endpoint is the 16-week PFS rate defined as the proportion of subjects that remain progression-free at week 16 by mRECIST v1.1.
Pharmacodynamic Evaluations	Biological markers of response will be assessed and will include examinations of cytokine and breast cancer-associated biomarker (eg, MUC-1) levels. For certain participating sites, additional immunological analyses will be performed. Please refer to Appendix 6.
Pharmacokinetic Evaluations	INXN-1001 PK will be assessed for subjects in in Part 1 Cycle 1 only.
Statistical Considerations / Analyses	This study is designed to evaluate the safety and efficacy of Ad-RTS-hIL-12 monotherapy or in combination with palifosfamide as determined by the 16-week PFS rate, defined as the proportion of subjects that survive progression-free at week 16. Statistical analysis will be performed separately for each study arm. Demographics and baseline characteristics will be summarized by treatment regimen. Primary Endpoints: • Safety and tolerability of therapy • 16-week PFS rate Secondary Endpoints: • Estimated PFS, duration of response, ORR, CBR Sample Size: The study will have 2 study arms and include a safety run-in as Part 1. Part 2 will have 2 study arms and utilize an adaptive Simon 2-stage design. Progressive disease will be assessed by mRECIST v1.1 at week 12, week 16, and at the Follow-up Tumor Assessment (week 24). **Treatment Arm A: Ad-RTS-hIL-12 Monotherapy** Part 1 will explore the safety Ad-RTS-hIL-12 monotherapy in 6 subjects, after 1 Cycle of therapy. During Part 2 for efficacy assessments, assume a targeted proportion of subjects who survive progression-free at week 16 of 35% with a proportion of no interest of 15%. a) Stage 1: if ≥1 subject of the first 6 subjects survives progression-free at week 16, add 7 subjects (n=13) to complete Stage 1 b) Stage 2: if ≥3 of 13 subjects survive progression-free at week 16 in Stage 1, add 20 subjects (n=33) to complete Stage 2 c) Possible Confirmatory Trial: if ≥9 of the total of 33 subjects survive progression-free at week 16, consider confirmatory trial

	Treatment Arm C: Combination Therapy Part 1 will explore the safety of combination therapy in 3 subjects after 1 cycle of therapy. During Part 2 for efficacy assessments, assume a targeted proportion of subjects who survive progression-free at week 16 of 65% with a proportion of no interest of 40%. a) Stage 1: add 9 subjects (n=12) to complete Stage 1 b) Stage 2: if ≥6 of 12 subjects survive progression-free at week 16 in Stage 1, add 14 subjects (n=26) to complete Stage 2 c) Possible Confirmatory Trial: if ≥15 of the total of 26 subjects survive progression-free at week 16, consider confirmatory trial The tumor responses will be assessed by mRECIST v1.1.

SCHEDULE OF STUDY PROCEDURES AND ASSESSMENTS

Table 2A: Part 1, Cycle 1 ONLY [contains PK schedule]

Activity	Screening[1] Day -28 to -1		Part 1 Cycle 1 ONLY					
	Pre-Registration	Post-Registration	Day 1	Day 2	Day 3	Day 7	Day 8	Day 15 ± 2 days
Clinical Assessments								
Informed Consent[2]	X							
Medical/Cancer History[3]	X							
Physical Exam[4]	X		X					
ECOG PS[5]	X		X					
Height	X							
Weight	X		X					
Vital Signs[6]	X		X	X	X	X		X
Adverse Events[7]	X	→	→	→	→	→	→	→
Concomitant Med.[8]	X	→	→	→	→	→	→	→
Clinical Laboratory								
Pregnancy test[9]	X		X					
Hematology tests[10]	X		X			X		X
Serum Chemistry tests[11]	X		X			X		X
Urinalysis[12]	X		X					X
ECG[13]	X		X					
Subject Registration[14]	X							
Pharmacokinetic Analysis[a]								
INXN-1001			X	X		X	X	
Tumor Response/ Pharmacodynamic								
Imaging Studies/Tumor Assessment[15]	X							
Serum cytokine profile & breast CA biomarkers[16]		X			X			X
Digital photography[17]		X						

Activity	Screening[1] Day -28 to -1		Part 1 Cycle 1 ONLY					
	Pre-Registration	Post-Registration	Day 1	Day 2	Day 3	Day 7	Day 8	Day 15 ± 2 days
Study Drug Administration								
Intratumoral INXN-2001[18]			X					
Oral INXN-1001[19]			←	—	X	—	→	
Verify Adherence to INXN-1001 dosing[19]						X		
Palifosfamide (IV), Arm C[20]			X	X	X			

[a] **PK Sampling Schedule**

Samples for PK analysis of INXN-1001 for Part 1 CYCLE 1 ONLY should be obtained on the following days and time points:

Day 1: **[6 samples]** Pre-dose (≤30 minutes prior to INXN-1001 dosing), 0.5, 1, 2, 4, 6 hours post-INXN-1001 dosing
Day 2: **[1 sample]** Pre-dose (≤30 minutes prior to INXN-1001 dosing)
Day 7: **[3 samples]** Pre-dose (≤30 minutes prior to INXN-1001 dosing), 1-2 hours, 4-6 hours post-INXN-1001 dosing
Day 8: **[1 sample]** 24 ± 3 hours post-Day 7 INXN-1001 dose

Table 2A numbered footnotes found after Table 2B (p.19)

Table 2B: ALL Cycles (EXCEPT for Part 1, Cycle 1 - see Table 2A)

Activity	Screening[1] Day -28 to -1		ALL Cycles (EXCEPT for Part 1 Cycle 1 - see Table 2A)					Post-Treatment Safety Assessment Visit[22]	Follow-Up Tumor Assessment Visit[23]
	Pre-Registration	Post-Registration	Day 1[21]	Day 2	Day 3	Day 7	Day 15 ± 2 days	28 ± 3 days post-last dose study drug	35 ± 7 days after PTSA visit
Clinical Assessments									
Informed Consent[2]	X								
Medical/Cancer History[3]	X								X[24]
Physical Exam[4]	X		X					X	
ECOG PS[5]	X		X					X	
Height	X								
Weight	X		X					X	
Vital Signs[6]	X		X	X	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			X	X	X	
Serum Chemistry tests[11]	X		X			X	X	X	
Urinalysis[12]	X		X				X	X	
ECG[13]	X		X					X	
Subject Registration/ Randomization[14]	X								
Tumor Response / Pharmacodynamic									
Imaging Studies/Tumor Assessment[15]	X		X C5			X C6			X
Serum cytokine profile & breast CA biomarkers[16]		X	X C5	X C1, C3, C6	X C1, C3, C6	X C1, C3, C6	X C1, C3, C6	X	
Digital photography[17]		X	X C5			X C2, C6		X	X

Activity	Screening[1] Day -28 to -1		ALL Cycles (EXCEPT for Part 1 Cycle 1 - see Table 2A)					Post-Treatment Safety Assessment Visit[22]	Follow-Up Tumor Assessment Visit[23]
	Pre-Registration	Post-Registration	Day 1[21]	Day 2	Day 3	Day 7	Day 15 ± 2 days	28 ± 3 days post-last dose study drug	35 ± 7 days after PTSA visit
Study Drug Administration									
Intratumoral INXN-2001[18]			X						
Oral INXN-1001[19]			←	—	X	—	→		
Verify Adherence to INXN-1001 dosing[19]						X			
Palifosfamide (IV) Arm C[20]			X	X	X				

Footnotes are provided on the following pages.

Tables 2A & 2B 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-specific 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 Days 1, 2, and 3 of each cycle, vital signs will be recorded prior to study drug dosing. Blood pressure should be monitored closely, with hydration as needed, to prevent hypotension after administration of INXN-1001. [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 case report form (CRF); AEs/SAEs that occur prior to informed consent should be added to the medical history 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 anticancer 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 or serum pregnancy test on the first day of each treatment cycle, prior to administration of any study drug.
10. Hematology tests include: complete blood count (CBC) and white blood cell (WBC) count, differential white blood cell count, red blood cell (RBC) 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 (eGFR), total bilirubin, total protein, albumin, blood urea nitrogen (BUN), 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, and prior to dosing of INXN-1001 and/or INXN-2001 injection and/or palifosfamide infusion on Day 1 of each cycle, and at the Post-Treatment Safety Assessment visit.
14. Centralized registration of eligible subjects will be completed according to a process defined by the Sponsor. During Part 1 of the study, subjects will be registered by the Medical Monitor to one of the treatment arms; during Part 2, subjects will be randomly assigned to a treatment arm.
15. 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
    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

    All imaging should be of diagnostic quality and include IV contrast. PET imaging is an acceptable alternative modality provided the CT component is of diagnostic quality as per RECIST 1.1 guidelines.

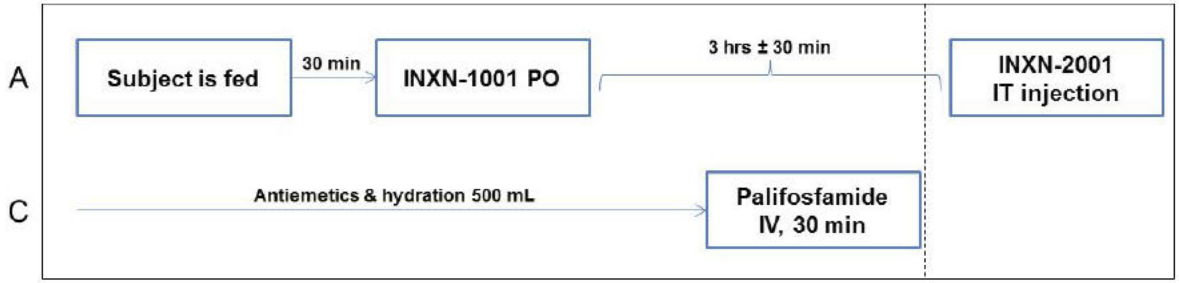
    For subjects with measurable lesions, target lesions should be selected and measured as per mRECIST v1.1 guidelines. (Appendix 4) Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be assessed qualitatively as non-target lesions.

    Disease sites are to be imaged throughout the study using the same method(s) used at screening. Chest and abdomen imaging are 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.

    Tumor response assessments will occur at 12 weeks and 16 weeks (C5D1 and C6D7), and the Follow-up Tumor Assessment visit (24 weeks) for all subjects, including those who may have experienced a dose delay. In the setting of this immunotherapy, a progression observed at week 12 requires confirmation at week 16. Additional tumor response assessments may occur at the discretion of the investigator.
16. A blood sample(s) for cytokine profiling, breast cancer-associated biomarker levels (eg, MUC-1), and also INXN-1001 levels. Please refer to the Laboratory Manual for details regarding sample processing and shipment.

    Screening (post-registration)
    Cycle 1 - Days 2, 3, 7, and 15
    Cycle 3 - Days 2, 3, 7, and 15
    Cycle 5 - Day 1 (to coincide with the tumor assessment)
    Cycle 6 - Days 2, 3, 7, and 15
    Post-treatment Safety Assessment

17. Digital photograph(s) of all visible injected and non-injected tumor(s), and of any visible local reactions in or around the injected lesion(s). Photographs are required post-registration, Cycle 2 Day 7, Cycle 5 Day 1, Cycle 6 Day 7, Post-Treatment Safety Assessment, and Follow-Up Treatment Assessment visits, and ad hoc as the investigator deems necessary. Important guidelines regarding photographic methodology will be provided in a separate manual.
18. Intratumoral INXN-2001 [redacted] injection should be administered 3 hours ± 30 minutes after the INXN-1001 dose. [redacted] 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[redacted]
19. The subjects will be dispensed INXN-1001 for self-administration after Day 1. The first INXN-1001 dose will be given at the clinical site in a fed state (within approximately 30 minutes after the start of a normal meal). The remaining 6 doses can be self-administered at the same time as on Day 1 (±1 hour) in the fed state. Study sites must verify adherence with INXN-1001 dosing. Subjects should return bottles of INXN-1001 to the clinical site to determine extent of subject adherence to self-administration preferably on Day 7.
20. The following temporal sequences of drug administration for Arm A monotherapy and Arm C combination therapy will be followed:



A = Ad-RTS-hIL-12 monotherapy
C = Combination of A + palifosfamide

For subjects enrolled in Arm C Ad-RTS-hIL-12 + palifosfamide Combination Therapy, the sequences for monotherapy + palifosfamide (sequences for Arms A + C) will be followed concurrently as shown above.

21. Day 1 clinical laboratory assessments may be performed within 15 days of the previous cycle through the Day 1 (prior to dosing) clinic visit. Clinical laboratory tests drawn for analysis prior to dosing on Day 15 of each cycle may be used for determining if the re-treatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration. Repeat assessments may be obtained for those subjects with parameters ≥Grade 3.

22. Post-Treatment Safety Assessment visit will be performed 28 ± 3 days after the last dose of study drug or prior to receiving another anticancer therapy.
23. 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.

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

3. INTRODUCTION

3.1 Disease Background (Recurrent/Metastatic Breast Cancer)

It is estimated that there are nearly 3 million women living in the United States with a history of invasive breast cancer, and an additional 226,870 women will be diagnosed in 2012.[1] Approximately 50% of women diagnosed with primary breast cancer will eventually relapse and develop metastatic or advanced disease. In addition, around 10% of patients present with metastatic disease at first diagnosis. The 5-year relative survival rate for women diagnosed with localized breast cancer is 98.6%; survival declines to 83.8% for regional stage and to 23.3% for distant stage. In addition to stage, factors that influence survival include tumor grade, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) status.

Locoregional recurrence in breast cancer is an indicator of an aggressive tumor, and early recurrence carries a poor prognosis. Approximately 11 and 20 percent of patients treated with adjuvant therapies develop locoregional recurrence within 5 and 10 years, respectively.[2] If disease is localized, surgical excision can be attempted. Breast cancer recurrences after mastectomy pose a therapeutic challenge with few surgical options. Many patients have systemic disease either at the time of their cutaneous or other recurrence or soon after, some may manifest disease only at the dermal site of disease, and a few are cured with aggressive treatment. These lesions often are widespread throughout the chest wall or involve heavily irradiated tissue.

Recent advancements in targeted therapy and improvements in treatment have heralded a significant improvement in survival, but metastatic breast cancer remains essentially incurable. Advanced metastatic breast cancer may be treated with systemic therapy (chemotherapy, biological therapy, targeted therapy, hormonal therapy), local therapy (surgery, radiation therapy), or a combination of these treatments.[3] Guidelines from the National Comprehensive Cancer Network for treatment of women with a locoregional breast cancer recurrence suggest consideration of systemic therapy after locoregional management, but do not specify the type of therapy or the duration.[4] In case instant response is required, such as rapidly progressive, threatening disease or massive symptoms, combination cytostatic therapy is usually administered. Monotherapy is a choice for patients in whom a long-term stabilization of metastatic disease is the objective.

Almost all patients who have received first-line chemotherapy for their metastatic progression will relapse or progress and require subsequent treatment. For these patients requiring second- and subsequent-line therapy, the goals of treatment are to maintain a good quality of life and to prolong survival.[5] Combination therapy is a mainstay of anticancer treatment, with optimal combinations producing synergistic antitumor responses, achieved by combining agents with non-overlapping mechanisms of action and safety profiles.

The addition of systemic chemotherapy (palifosfamide) to immunotherapy (Ad-RTS-hIL-12) in patients who are treated for a recurrence or metastasis of breast cancer may decrease the risk of a subsequent local recurrence or distant metastasis. This combination of therapeutic

agents has not yet been studied in human subjects, particularly in this disease; therefore, the safety profile is not yet clearly defined. The Phase II study proposed in this protocol (ATI001-201) will therefore examine the safety, antitumor activity, and immunological effects of intratumoral (IT) injections of INXN-2001 + orally administered INXN-1001 (Ad-RTS-hIL-12 monotherapy) alone and in combination with palifosfamide in subjects with advanced breast cancer.

[redacted]

3.3 Background and Rationale for Treatment Strategy

3.3.1 IL-12 Immunotherapy

Local cytokine delivery to tumors can induce a number of antitumor responses that include: 1) direct suppression of tumor cell proliferation, 2) induction of apoptosis, 3) antiangiogenesis, and 4) enhancement of local antitumor immune response. This enhancement of local antitumor 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.[10]

Interleukin (IL)-12 is a potent immunostimulatory cytokine which activates and recruits dendritic cells (DC) that 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 NK and $CD8^+$ T cell cytotoxic activities, augmentation of antigen-specific antitumor responses, and the production of IFN-γ and TNF-α.[11-14] IL-12 also stimulates neutrophil and macrophage production of superoxides and nitric oxide, and induces the production of antiangiogenic factors by tumor cells. IL-12 has been shown to have antitumor activity in several animal models.

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), intraperitoneal (IP), or subcutaneous administration. [15-24] Systemic administration of IL-12 resulted in induction of systemic immune responses in some subjects, but there was limited clinical improvement and severe toxicity. In a Phase II trial evaluating repeated IV administrations of recombinant IL-12 in subjects with renal cell carcinoma, 12 of 17 subjects were hospitalized and 2 died due to hypovolemic shock. The study was halted, as most subjects experienced serious adverse events (SAEs) during the first cycle of treatment, and there was a high frequency of Grade 3 and 4 AEs, with 65% of subjects showing leukopenia and 47% showing hyperbilirubinemia and elevated aspartate transaminase (AST).[24] 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.

It is therefore important to achieve the immunological benefits that IL-12 provides while avoiding the toxicity elicited by systemic delivery. IT delivery of Ad-RTS-hIL-12 accomplishes precisely this objective by allowing adjustment of IL-12 gene transcription by varying the dose of INXN-1001. The vector is localized in the tumor and the gene is produced locally in response to predetermined doses of the oral ligand. In this scenario the dose can be lowered if any toxicities are observed.

3.3.2 Palifosfamide

Palifosfamide is a potent, bi-functional, DNA alkylating agent that has activity in multiple tumors by evading typical resistance pathways. Palifosfamide is in the same class as bendamustine, cyclophosphamide, and ifosfamide. Palifosfamide is the functional active metabolite of ifosfamide (IFOS); however, palifosfamide has not been shown to cause toxicities observed with IFOS metabolites acrolein or chloracetaldehyde, such as hemorrhagic cystitis or neuropathy. The initial clinical development of palifosfamide focused on assessing the safety and tolerability of palifosfamide when administered as a single agent to subjects with advanced solid tumors. This approach was followed by Phase I through Phase III studies of palifosfamide in combination with doxorubicin as a potential treatment for advanced soft tissue sarcomas. An additional combination chemotherapy regimen of palifosfamide with etoposide + carboplatin is also being evaluated as a potential therapy for subjects with small cell lung cancer.

Palifosfamide is currently being investigated in a Phase III clinical trial. The MATISSE trial is evaluating palifosfamide (130 mg/m^2 × 3 q21 days) in combination with carboplatin and etoposide as a treatment for subjects with extensive-stage small cell lung cancer.

[redacted]

3.4.2 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 subjects.

In a Phase I/II clinical trial in prostate cancer using 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.[26] Numerous studies have utilized adenoviral vectors to deliver the p53 gene via the IT route. Oral leukoplakia subjects 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 (DLT), and administration of the (rAd)-p53 was well tolerated.[27] No DLT was observed in chemoradiation-resistant advanced esophageal carcinoma subjects receiving IT injections of adenovirus containing p53 (10 x 10^{11} particles to 25 x 10^{11} particles).[28] 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 subjects) and local pain (30% of subjects.) Three subjects displayed hyperglycemia that was attributed to nutrition, 2 subjects showed hypocalcemia, and 1 subject each experienced partial thromboplastin time (PTT) elongation or an increase in serum amylase or creatinine. No other significant laboratory abnormalities were detected on follow-up evaluations.[28]

An adenoviral vector encoding hIL-12 has been studied using IT injection in subjects with advanced digestive malignancies, at doses up to 3 x 10^{12} vp, 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.[29]

3.4.3 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 subjects has proven to limit or abrogate the toxic effects of hIL-12, thus providing an effective way to provide this potent immunomodulatory cytokine. In Phase I and II clinical trials where IL-12 was delivered locally, increased systemic IL-12 serum levels post-baseline were not observed.[30-32] 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 AEs were observed in subjects treated at several dose levels. Similarly, IT injection of a plasmid DNA-encoding human IL-12 (2 to 20 mg of total DNA) in subjects with melanoma was also well tolerated.[34] Limited elevations in serum cytokines were observed after treatment. Intra-peritoneal (IP) injection of hIL-12 plasmid DNA formulated in polyethyleneglycol-polyethyleneimine-cholesterol 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.[38] Plasmid DNA was not detected in the subjects' 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 IT injection of adenoviral vectors encoding hIL-12 at doses ranging from 2.5 x 10^{10} to 3 x 10^{12} vp, to subjects with advanced pancreatic, colorectal, or primary liver malignancies.[29] Treatment was well tolerated and DLT was not reached. AEs were transient, with fever, malaise, sweating, and lymphopenia being the most commonly observed. No cumulative toxicity was observed. All AEs were deemed to be related to injection of the virus and not to transgene expression.

[redacted]

3.5 Rationale for Study Design and Dose Selection

The objectives of this Phase II clinical trial are to assess the safety and tolerability, PFS rate at 16 weeks, ORR, and immunological and biological effects of IT injections of INXN-2001 + INXN-1001 as monotherapy or in combination with palifosfamide. To provide an opportunity to monitor the safety of Ad-RTS-hIL-12 monotherapy and Ad-RTS-hIL-12 + palifosfamide (combination therapy) in subjects with breast cancer, a safety run-in will occur, with evaluation following one cycle of study drug.

The study will commence with Ad-RTS-hIL-12 monotherapy using a 140-mg dose of INXN-1001 administered daily Days 1-7 in 6 subjects (21-day cycle). Following a safety assessment, combination therapy of Ad-RTS-hIL-12 + palifosfamide (120 mg/m^2, Days 1, 2, 3 of a 21-day cycle, n=3) will commence. Safety in Part 1 of the study will determine commencement of randomization for Part 2.

[redacted]

During the conduct of this study, in addition to monitoring of safety and therapeutic efficacy, peripheral blood samples will be studied for indications of immunological and biological effects including analyses of breast cancer-associated cytokines/biomarkers. The combined clinical and laboratory data will be studied to assess for activity, and will be used in decision making for expansion of treatment arms.

The population for this study was selected as those subjects with recurrent or metastatic breast cancer (which is rarely curable with standard therapy) and accessible lesions, who are therefore considered to be appropriate candidates for clinical trials exploring new forms of treatment. Because breast cancers may be responsive to immunotherapeutic approaches (see Section 4.1 Disease Background), this subject population may potentially benefit from investigational therapy with Ad-RTS-hIL-12 alone or in combination with palifosfamide.

4. STUDY OBJECTIVES AND DESIGN

4.1 Objectives

The primary objectives are to:

- Assess the safety and tolerability of INXN-1001 + INXN-2001 (Ad-RTS-hIL-12) as monotherapy and in combination with palifosfamide in subjects with recurrent and/or metastatic adenocarcinoma of the breast and accessible lesions (ie, in the opinion of the investigator is/are palpable and/or injectable), which are not amenable to surgical resection or radiation with curative intent

- Assess the efficacy of repeated cycles of IT injections of INXN-2001 (Ad-RTS-hIL-12) with INXN-1001 (oral activator ligand) as monotherapy or in combination with palifosfamide as measured by the 16-week progression-free survival (PFS) rate, defined as the proportion of subjects who survive progression-free at week 16 in the study population

The secondary objectives are to:

- Estimate PFS by modified Response Evaluation Criteria in Solid Tumors (mRECIST) v1.1
- Assess ORR by mRECIST v1.1
- Assess duration of response
- Assess clinical benefit rate (CBR = proportion of subjects with complete response [CR], partial response [PR], or stable disease [SD]) by mRECIST v1.1
- Evaluate pharmacodynamic tumor markers (eg, breast cancer-associated biomarkers such as MUC-1)
- Assess the PK of INXN-1001 in subjects with recurrent and/or metastatic adenocarcinoma of the breast

4.1.1 Study Design and Treatment Arm Expansion Procedure

4.1.1.1 Overall Study Design

This is a randomized, open-label, Phase II study of Ad-RTS-hIL-12 monotherapy or Ad-RTS-hIL-12 in combination with palifosfamide in subjects with recurrent/metastatic breast cancer with accessible lesions.

Two treatment arms will be assessed for safety, followed by random assignment to Ad-RTS-hIL-12 monotherapy or combination therapy. Each subject will be treated for up to 6 treatment cycles, with each cycle being 21 days in duration.

Safety and tolerability will be assessed by the incidence and severity of AEs as determined by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03. A SRC comprised of the Medical Monitor, enrolling Principal Investigators (PIs), and Sponsor representatives, will be convened to review safety information and to decide upon further subject enrollment.

The antitumor activity of study treatment will be assessed according to mRECIST v1.1 guidelines.[31] (Appendix 4)

4.1.2 Treatment Parameters and Duration

Subjects will receive IT injections of INXN-2001 [redacted] on Day 1 of each cycle, will also receive oral doses of INXN-1001 (140 mg daily) for 7 days. Subjects enrolled in Arm C combination therapy will also receive palifosfamide (120 mg/m^2/day) by IV infusion over approximately 30 minutes on Days 1, 2, 3 of each cycle.

All subjects may receive up to 6 cycles of study treatment. Subjects receiving Ad-RTS-hIL-12 with disease progression (per mRECIST v1.1 criteria) may still complete the 6 treatment cycles, because tumor nodules may enlarge due to the anticipated induction of an inflammatory response, unless the progression is clinically significant in the opinion of the investigator (see Appendix 4). Furthermore, in studies with a diversity of immunotherapies in different cancer types, objective responses have been shown to occur after an increase in tumor burden characterized as progressive disease (PD) by World Health Organization or RECIST criteria.

4.1.3 Safety Review Committee (SRC)

An SRC comprised of the Medical Monitor, enrolling PIs, and Sponsor representatives will hold periodic teleconferences to evaluate the safety and treatment status of all subjects available during the Safety Run-in (Part 1) at several stages:

- after 6 subjects Arm A have completed the first cycle,
- after 3 subjects in Arm C have completed the first cycle,
- at any other time as needed following random assignment in Part 2.

The safety run-in of Arm C (n=3) will occur with a 1-week interval between dosing of subjects and an SRC will convene after all subjects have received 1 cycle of therapy. The SRC has the authority to recommend dose or regimen 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 appropriate.

Following a treatment arm review, the SRC may recommend proceeding with enrolling additional subjects in the current treatment arm or not enrolling any additional subjects.

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 at any time.

4.1.4 Enrollment Procedure

During **Part 1**, 6 eligible subjects will enroll into Arm A, followed by 3 subjects in Arm C.

During **Part 2**, eligible subjects will be randomly assigned by the Sponsor or its representative:

- Subjects will be adaptively randomized in a 1:1 ratio to receive Ad-RTS-hIL-12 monotherapy (Arm A) or combination therapy (Arm C) according to the current status of the treatment arms and whether or not a subject has consented to the "Additional Immunologic Assessments" until the desired number of subjects is fully enrolled in each study arm.

4.1.5 Treatment Arm Expansion Procedure

Should a subject discontinue study treatment before receiving 6 cycles for reasons other than progression, another subject may be added to the treatment arm to allow for full analysis. Response evaluation and criteria for futility are described in Section 9 Statistical Methods.

Subject enrollment and expansion of treatment Arms A and C will proceed according to a standard Simon 2-stage design (Table 1). An alternate dose level of palifosfamide and/or INXN-1001 may be explored after review of all available safety and activity data, and will be communicated by an administrative letter and enacted upon issuance.

Table 1 Study Design (see Protocol Synopsis)

Part	Treatment Arm	Intervention (21-day cycle)
1 Safety Run-in	A	Ad-RTS-hIL-12 monotherapy (n=6) *[INXN-1001 (140 mg daily)+ INXN-2001* [redacted]
	C	Combination therapy; n=3 *[INXN-1001 (140 mg daily) + INXN-2001* [redacted] *+ palifosfamide (120 mg/m^2)]*
2 Randomization	A	Ad-RTS-hIL-12 monotherapy *[INXN-1001 (140 mg daily) + INXN-2001* [redacted]
	C	Combination therapy *[INXN-1001 (140 mg daily) + INXN-2001* [redacted] *+ palifosfamide (120 mg/m^2)]*

5. SUBJECT SELECTION

The target population for this study is adult subjects with recurrent and/or metastatic adenocarcinoma of the breast with accessible tumors (eg, in the opinion of the investigator is/are palpable and/or injectable) not amenable to surgical resection or radiation with curative intent.

5.1 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. Histologically or cytologically confirmed adenocarcinoma of the breast, either locally recurrent or metastatic disease with injectable lesions, for which no proven curative therapy exists. Locally recurrent disease must not be amenable to surgical resection or radiation with curative intent.
3. Subject has failed or progressed on at least 1 prior systemic chemotherapy regimen ± biologic/experimental therapy (if first-line therapy, failure or progression during or within the first 30 days).
4. Resolution of all chemotherapy or radiation-related toxicities to Grade 1 severity or lower, except for stable sensory neuropathy ≤ Grade 2 and alopecia.
5. A minimum of 2 lesion(s) assessed by imaging using mRECIST v1.1. 1 or more lesions NOT to be injected AND at least 1 injectable lesion(s) (eg, may use radiographically-guided injection of lesions if available at site)
6. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, 2
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. Women of childbearing potential (perimenopausal

women must be amenorrheic for at least 12 months to be considered of non-childbearing potential) must have a negative pregnancy test at screening.

8. Adequate bone marrow reserve as indicated by:
    a) Absolute neutrophil count (ANC) > 1500/μL (without use of growth factors within 7 days)
    b) Absolute lymphocyte count (ALC) > 700/μL (without use of growth factors within 7 days)
    c) Platelet count > 100,000/mm^3 (without transfusion in prior 7 days)
    d) Hemoglobin > 9.0 g/dL (without transfusion in prior 7 days)
9. Adequate renal function as evidenced by eGFR using the Modification of Diet in Renal Disease (MDRD) equation: eGFR ≥ 60 mL/min/1.73 m^2
10. Adequate liver function as evidenced by the following:
    a) Bilirubin ≤ 1.5 times the upper limits of normal (ULN)
    b) ALT and AST ≤ 2.5×ULN, in the case of liver metastases ≤ 5×ULN

5.2 Exclusion Criteria

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

1. Subjects with *HER2/neu*-positive immunohistochemistry 3+ or fluorescence in situ hybridization-amplified breast tumors who are eligible for, but who have not received HER2-targeted therapy (eg, trastuzumab)
2. Concomitant anticancer therapies (eg, endocrine therapy for breast cancer)
3. Prior therapies discontinuation periods:
    a) Radiation within 3 weeks of enrollment
    b) Chemotherapy within 4 weeks of enrollment
    c) Nitrosoureas within 6 weeks of enrollment
    d) Biologic therapy and/or immunomodulatory therapy (eg, granulocyte colony stimulating factor [G-CSF]/granulocyte macrophage colony stimulating factor [GM-CSF], interferons or interleukins, growth hormone, intravenous immunoglobulin [IVIG], retinoic acid), check point inhibitors (eg, ipilimumab, anti-PD-1 or anti-PDL-1 antibodies) within 6 weeks of enrollment
    e) No washout period is required for endocrine therapy
4. Radiation therapy encompassing >25% of bone marrow
5. History of bone marrow or stem cell transplantation
6. Any congenital or acquired condition leading to an inability to generate an immune response, including concomitant immunosuppressive therapy
7. Immunosuppressive therapy:

a) Systemic immunosuppressive drugs including corticosteroids (prednisone equivalent >10 mg/day, http://www.medcalc.com/steroid.html) within 6 weeks
b) Immune suppression/requiring immunosuppressive drugs, including subjects with organ allografts
c) Active autoimmune disease requiring the equivalent of >10 mg/day of prednisone

8. Major surgery within 4 weeks of study treatment, or major surgery planned for duration of study participation
9. History of prior malignancy, unless the prior malignancy was diagnosed and definitively treated ≥5 years previously with no subsequent evidence of recurrence

   NOTE: This does not apply to previous breast cancer, carcinoma in situ of the cervix, inactive melanoma or non-melanoma skin cancer, or Grade 1 papillary bladder cancer
10. Subjects with brain or subdural metastases are not eligible, unless local therapy completed and corticosteroids discontinued for this indication for ≥4 weeks before starting study treatment. Any signs (eg, radiologic) and/or symptoms of brain metastases must be stable for ≥4 weeks before starting study treatment; radiographic stability should be determined by comparing a contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) brain scan performed during screening to a prior scan performed at least 4 weeks earlier.

    NOTE: 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.
11. Any medications that induce, inhibit or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of study drug
12. Subjects with meningeal carcinomatosis
13. Known significant hypersensitivity to study drugs or excipients
14. History of malabsorption syndrome or other condition that would interfere with enteral absorption
15. International Normalized Ratio (INR) and activated 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
16. New York Heart Association (NYHA) Class II or greater congestive heart failure OR active ventricular arrhythmia requiring medication
17. Any other unstable or clinically significant concurrent medical condition (eg, infection requiring systemic anti-infective agents) that would, in the opinion of the investigator, jeopardize the safety of a subject and/or their compliance with the protocol
18. Localized infection at site of injectable lesion(s) requiring anti-infective therapy within 2 weeks of the first dose of study drug

NOTE: Appropriate testing (eg, punch biopsy [PBx] with bacterial counts) must be performed to rule out infection in cases where the presence of infection is ambiguous or clinical signs of infection are evident.

5.3 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:

- 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 total 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 treatment-related AE meeting withdrawal criteria.
- Substantial noncompliance with the requirements of the study.
- Subjects with a confirmed positive pregnancy test.
- 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 AEs in order to determine the outcome of the event.

5.4 Replacement of Subjects

Should a subject discontinue study treatment before receiving 6 cycles for reasons other than progression, another subject may be added to that study arm for full analysis.

5.5 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 institutional review board (IRB)/independent ethics committee (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.

6. TREATMENT PROCEDURES

6.1 Screening

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.

6.1.1 Informed Consent

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

6.1.2 Inclusion and Exclusion Criteria

Inclusion and exclusion criteria will be reviewed for each potential subject and documented in the case report form (CRF).

6.1.3 Registration/Randomization of Subjects

Centralized registration/randomization of subjects will be completed according to a process defined by the sponsor (see Section 5.1.4). Each subject will be assigned a unique identification number. Once assigned, a subject's identification number will not be reused.

6.2 Study Drugs

6.2.1 Accountability and Dispensation

The 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 United States Food and Drug Administration (US FDA) Form 1572, and may only be dispensed by authorized personnel at the institution(s) listed therein.

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

6.2.2 Handling and Storage

Study drugs must be stored in a restricted access area under the storage conditions indicated in the Investigator's Brochure(s) or Pharmacy Manual.

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

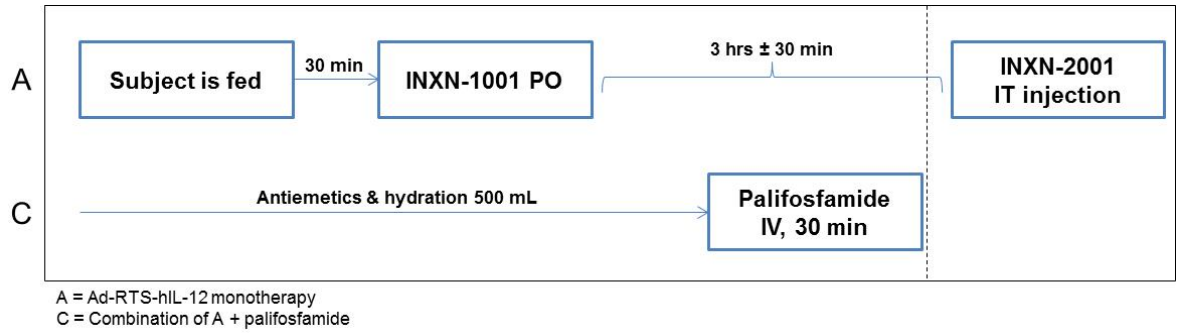
6.2.3 Treatment Regimen

A combination of up to 3 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 (once daily doses on Days 1-7) in combination with an IT injection of INXN-2001 (on Day 1 of each cycle). For each 21-day treatment cycle, INXN-2001 dosing will occur 3 hours ± 30 minutes after the first dose of INXN-1001. INXN-2001 will be given as IT injections of approximately [redacted] 1 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 an alternate lesion or a draining, pathologic lymph node of a previously accessible lesion. A pathologic lymph node must be defined as a lesion per mRECIST (Appendix 4). 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 must be instructed to maintain good oral hydration on and between dosing days. [redacted] For treatment Arm C Combination Therapy (Ad-RTS-hIL-12 + palifosfamide), palifosfamide will be administered following administration of INXN-1001 as a 30-minute IV infusion, and on Day 1 of each cycle the infusion should complete before injection with INXN-2001.

Palifosfamide will be administered intravenously at a dose of 120 mg/m^2/day as a 30-minute infusion on Days 1, 2, and 3 of a 21-day cycle for up to 6 cycles.

The temporal sequence of the treatment regimens for study Arms A and C are shown in Figure 4. For subjects enrolled in Arm C Ad-RTS-hIL-12 + Palifosfamide Combination Therapy, the sequence of regimens A + palifosfamide will be followed concurrently as shown below.

Figure 4. Study Arm Treatment Regimens



6.2.4 Preparation and Administration of Study Drug

Please refer to the Pharmacy Manual for additional information.

6.2.4.1 Preparation and Administration of INXN-1001

INXN-1001 will be provided by the Sponsor as capsules.

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 (within approximately 30 minutes after the start of a normal meal).

6.2.4.2 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 each of the remaining treatment period, and will be reminded to take their dose via a phone call when they will not have a clinic visit.

Subjects should be instructed not to make up for missed doses.

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.

6.2.4.3 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 IT 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 determination of a path forward. Lesions displaying signs of localized infection should not be injected.

Note: In the absence of the availability of radiographically-guided injections, one accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-2001.

The INXN-2001 IT administration should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion or pathologic lymph node to reach all aspects of the entire lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins.

Should the tumor selected for injection not support the entire INXN-2001 injection volume (approximately 1 cc), another tumor should be injected with the remaining volume to ensure that all subjects receive approximately [redacted]. If another tumor is not available, then the remaining volume should be injected into a draining, pathologic lymph node of the injected tumor. At any cycle, if no accessible lesion for INXN-2001 injection is present (eg, due to complete resolution), then INXN-2001 should be injected into a draining, pathologic lymph node of a previously accessible lesion. A pathologic lymph node must be defined as a lesion per mRECIST (Appendix 4).

NOTE:

- All subjects receiving INXN-2001 must be adequately hydrated with approximately 2000 mL per 24 hours of oral electrolyte solution for 72 hours during Cycles 1 and 2. Subjects 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. The subject should be instructed to call the clinical site if any such reactions develop or do not resolve within 24 to 48 hours.

6.2.4.4 Preparation and Administration of Palifosfamide

Palifosfamide should be administered IV at a dose of 120 mg/m^2/day, as an approximate 30-minute infusion on Days 1, 2, and 3 of a 28-day cycle, for up to 6 cycles. Palifosfamide is supplied in single-use glass vials each containing 350 mg of active product. Each vial is to be reconstituted with sodium chloride injection, USP, to a final concentration of 50 mg/mL and appropriate dose transferred into a 250 mL 0.9% sodium chloride IV infusion bag.

Note: Total cumulative time from reconstitution in vial through completed dose administration should not exceed 2.5 hours (150 minutes).

Information regarding the preparation of the palifosfamide dose will be provided in the Pharmacy Manual. The dose (mg) of palifosfamide should be calculated based on the subject's actual body weight as measured on Day 1 of each cycle. Body surface area (BSA) may be calculated as per the standard practice at each investigative center; however, the same formula should be used throughout the study when calculating a subject's BSA. The palifosfamide dose must be prepared as described in the Pharmacy Manual, and administered within permitted time window allowed for reconstitution in vial, dilution in the IV infusion bag, and through completion of the IV infusion.

NOTE:

- All subjects receiving palifosfamide must be adequately hydrated with at least 500 mL of fluids (oral or IV as appropriate), in addition to the infusion administered, on each day of palifosfamide dosing. Subjects should be instructed to maintain good oral hydration on and between dosing days.
- Electrolytes and hematologic toxicity need to be carefully monitored during clinical studies with palifosfamide.

Study treatment should be administered according to the planned 21-day treatment cycle, with treatment delays allowed for study-drug related toxicity as described in Section 7.2.5. If the Day 1 visit for a re-treatment cycle is delayed for other reasons (eg, due to inclement weather), then the visit should be rescheduled to occur as soon as possible. If any non-prolonged delay for other reasons is to occur, the Medical Monitor should be consulted.

A missed Day 2 or Day 3 study drug administration may be rescheduled to Day 4 of the same treatment cycle. If both Day 2 and Day 3 are missed, then only a single day may be rescheduled to Day 4 and the other will be skipped. In either case, the total duration of the treatment cycle should remain 21 ± 1 day by shortening the rest interval by 1 day. If the missed dose cannot be administered on Day 4, then it must be skipped and the subject should continue on protocol and take the next dose as originally planned.

6.2.5 Retreatment Criteria, Dose Delays and Modifications

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

Hemoglobin ≥ 9 g/L	eGFR ≥ 60 mL/min/1.73 m^2
Lymphocytes > 700/ mm^3	AST and ALT ≤ 2.5 x ULN For subjects with documented liver metastases, ALT and AST ≤ 5×ULN
Neutrophils ≥ 1,500/ mm^3	Total bilirubin < 1.5 x ULN
Platelets ≥ 100,000/ mm^3	INR and PTT <1.5 x ULN

Clinical laboratory tests drawn pre-treatment for analysis on Day 1 of each cycle may be used for determining if the re-treatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration. Two treatment delays for recovery to acceptable levels are allowed at weekly intervals up to a maximum of14 days. In the event that a treatment-related dose delay is necessary for palifosfamide, then the next cycle of study treatment will be administered at 80% of the dose (Table 3). If an AE is clearly related to 1 study drug and not the other (in combination Treatment Arm C), then dose modification/delay is to occur for the relevant study drug (INXN-1001 and/or palifosfamide). A maximum of two dose reductions, of either INXN-1001 or palifosfamide, will be allowed.

Table 3. Dose Modifications for Palifosfamide-related Toxicity[a]

ADVERSE EVENT	PALIFOSFAMIDE
Hematologic	
Platelet count <100 × 10^9/L	Delay dose of palifosfamide up to 14 days until platelet count ≥ 100 X 10^9/L, then reduce by 20%.
ANC < 1500/mm^3	Delay dose of palifosfamide up to 14 days until ANC returns to ≥ 1500, then maintain dose when restarted.
Neutropenic fever	Delay dose of palifosfamide up to 14 days until resolved, then reduce by 20%.
Any Grade 4 hematologic toxicity	Delay dose of palifosfamide up to 14 days until Grade ≤1 or baseline, then reduce by 20%.
Estimated Glomerular Filtration Rate (eGFR) (Appendix 5)	
≥25% up to <50% decrease from baseline[b]	Hold dose until repeat test is performed. Reduce dose of palifosfamide by 20% if confirmed on repeat test.
≥50% decrease from baseline[2]	Hold dose until repeat test is performed. Discontinue palifosfamide if confirmed on repeat test.
Non-hematologic (other than creatinine or urinary electrolyte wasting)	
Not treatment-related Grade 3 if unresponsive to appropriate therapy.	Delay dose of palifosfamide for up to 14 days until Grade ≤1 or baseline, then reduce by 20%.
Not treatment-related Grade 4	Delay dose of palifosfamide up to 14 days until ≤ Grade 1 or baseline, then reduce by 20%.

[a]When indicated, the maximum permitted delay of study drug administration due to toxicity and/or for confirmatory laboratory testing is 14 days.

[b]Baseline eGFR determined based on the most recent serum creatinine measurement prior to study drug administration (ie, Cycle 1 Day 1 or Screening).

Subjects who experience a treatment-related Grade 4 AE, other than nausea and/or vomiting in the absence of optimal treatment with antiemetics, will be discontinued from study treatment. If, however, the subject has experienced a substantial benefit from treatment and the PI and Medical Monitor recommend continuation on the study, re-exposure to the experimental treatment may proceed on a case-by-case basis.

6.2.6 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 NCI CTCAE v4.03.

Table 4 outlines the injection site reaction grading system, associated symptoms, and the recommended actions to be taken. 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 4. Injection Site Reaction Severity Grading and Management

CTCAE Grade	Symptoms	Course of Action
1	Tenderness with or without associated symptoms (e.g., 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. If toxicity meets seriousness criteria, immediately report as per SAE reporting procedures; see Section 8.4.
4	Life-threatening consequences; urgent intervention indicated	Permanently discontinue study treatment and notify Medical Monitor. Defaults to "serious". Immediately report as per SAE reporting procedures; see Section 8.4.
5	Death	Immediately notify Medical Monitor and report as per SAE reporting procedures; see Section 8.4. Discontinue further subject enrollment.

As with all AEs, injection-related signs or symptoms which, in the Investigator's judgment, are not related to immunologic reactions 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.

6.2.7 Prophylactic Antipyretic and/or Analgesic Administration

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

> **NOTE**: Since fever and flu-like symptoms (eg, fever, headache, chills, dehydration, etc.) are commonly experienced following adenoviral vector administration, it is highly recommended that subjects be treated with prophylactic, antipyretic and/or analgesic medications prior to study drug administration and the first 72hr of each cycle.

Please refer to Appendix 3 for the recommended regimen for the prophylactic administration of antipyretics and/or analgesics.

6.2.8 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 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.

6.3 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.

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. (except those listed Appendix 2)
- 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 anticancer therapy (chemotherapy, radiotherapy, etc.) while receiving study treatment.

6.4 Evaluation and Assessment Procedures

6.4.1 Schedule of Assessments

A tabular schedule of evaluations and procedures is provided as Schedule of Study Procedures and Assessments following the Protocol Synopsis in Section 3.

6.4.2 Safety Evaluations

6.4.2.1 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.

6.4.2.2 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 on Day 1 of each cycle.

6.4.2.3 ECOG Performance Status

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

6.4.2.4 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.

6.4.2.5 Vital Signs

Vital signs will include blood pressure, pulse rate, temperature, and respiration rate. Subject's blood pressure is to be monitored closely, with hydration as needed to prevent hypotension for 72 hours after administration of INXN-2001. Blood pressure assessment is required on Days 1, 2, 3 for Cycle 1 and Cycle 2. [redacted]

6.4.2.6 Adverse Events

Monitoring and recording of AEs and 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 8.0.

6.4.2.7 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.

6.4.2.8 Hematology

Hematology tests include: complete blood count (CBC) and white blood cell (WBC) count, differential WBC count, red blood cell (RBC) count, hematocrit, hemoglobin, RBC indices, reticulocyte count, MCV (mean corpuscular volume) and platelet count. PTT and INR will also be evaluated.

6.4.2.9 Serum Chemistry

Serum chemistry tests include: AST, ALT, LDH, ALP, creatinine, total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, sodium, potassium, chloride, calcium, phosphorus and bicarbonate. The eGFR will be calculated from the serum creatinine at each chemistry time point using the MDRD equation (Appendix 5); in cases where the MDRD equation may not be suitable, a 24-hour urine creatinine clearance test may be substituted with prior approval of the Medical Monitor.

6.4.2.10 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.

6.4.2.11 Electrocardiogram (ECGs)

A standard, single, 12-lead ECG for local safety assessment will be performed at screening, 2.5 to 3 hours after INXN-1001 dosing (and prior to palifosfamide infusion and 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.

6.4.2.12 Immune Response Analyses

Plasma and/or serum cytokine levels will be assayed using enzyme-linked immunosorbant assay (ELISA) methodology. One or more serum biomarkers (eg, MUC-1) or cytokines will be assessed.

6.4.2.13 INXN-1001 Pharmacokinetic Assessment

INXN-1001 PK assessment will occur for subjects enrolled in Part 1 during Cycle 1 of study treatment. Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day 8; as defined in Table 2A, Schedule of Assessments. INXN-1001 levels will also be assessed using the samples drawn for cytokine analysis.

6.4.3 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
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 each subject, disease sites are to be assessed throughout the study using the same method(s) of assessment used at screening. Chest and abdomen imaging are 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.

Target and non-target lesions should be selected and measured as per mRECIST v1.1 guidelines[39] (see Appendix 4). Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be assessed as non-target lesions. For each subject, disease sites are to be assessed throughout the study using the same method(s) of assessment used at screening. Tumor response assessments will occur at 12 weeks, 16 weeks, and the Follow-up Tumor Assessment visit (24 weeks) for all subjects, including those who may have experienced a dose delay. Additional tumor response assessments may occur at the discretion of the investigator.

The investigator will evaluate each subject for response to therapy according to mRECIST v1.1 guidelines.

In the setting of this immunotherapy, a progression observed ≤ week 12 requires confirmation at week 16 (see Appendix 4 for further details). 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 anticancer therapy has been initiated, or up to 1 year, 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 and non-injected tumors, and of any remaining visible local reactions in or around the injected lesion(s). Important guidelines regarding photographic methodology will be provided in a separate manual.

6.4.4 For Participating Sites: Assessments of Transgene Function, Immunological Activities and Biological Effects (please refer to Appendix 6)

6.4.4.1 Transgene Function

For subjects receiving Ad-RTS-hIL-12, punch biopsies (PBx) or fine-needle aspirates (FNA) 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 IL-12, and expression of IL-12-induced downstream genes. Expression of IL-12 and IL-12-induced genes will be evaluated via qRT-PCR, using a panel of primers to known IL-12 regulated genes in breast cancer as well as signaling pathways downstream from IL-12.

For all subjects, FNAs of the tumor(s) and/or associated tumor-involved draining lymph nodes will be collected for immunohistochemistry (IHC).

If a non-injected distal lesion responds to treatment, then an FNA will be obtained to assess the presence of the adenoviral vector and evaluate the mechanism of the response.

6.4.4.2 Immunological Activities

Part of the biopsied tumor(s) will be evaluated by IHC to assess cellular infiltration in the tumor. Cellular infiltration by effector cells, such as T cells and their subsets CD4+, CD8+, and CD56, will be evaluated as well as immune suppressor elements, such as T-regulatory cells (Tregs). Biopsy specimens will also be evaluated for markers of immune activation. Blood will be drawn for assessing cell-mediated immunity, especially frequency of cytotoxic T lymphocytes (CTLs) and Tregs. PBMCs incubated with a pool of breast cancer antigen-derived peptides will be assayed by ELISPOT and/or flow cytometric intracellular staining for relevant cytokines to enumerate the anti-breast cancer CTLs.

6.4.4.3 Other Biological Effects

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. [redacted] An analysis of tumor infiltrates will be performed by IHC with Abs to different immune cell population markers such as CD3, CD4, CD8, CD25, FOX-P3, CD56, etc.

7. ADVERSE EVENTS

7.1 Adverse Event (AE) Definition

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related. Any worsening of a pre-existing 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 pre-existing 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 AEs.
- In the event that a constellation of symptoms results in a confirmed diagnosis, the diagnosis (not the symptoms) should be recorded as the AE term.
- If a diagnosis cannot be established, the symptoms should be recorded as the AE(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.

7.2 Evaluation of Adverse Events (AEs)

7.2.1 Determination of Seriousness

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

7.2.1.1 Serious Adverse Event (SAE)

An AE is considered an SAE if at least 1 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 subject, 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 subject 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 subject 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 an SAE. (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.

7.2.1.2 Non-Serious Adverse Event

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

7.2.2 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 subject, 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 subject'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 subject is at immediate risk of death.
- Death (Grade 5): The subject 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, 1 start date and stop date should be recorded along with the maximum intensity experienced for that event over that 3-day timeframe).

7.2.3 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.

7.3 Documenting Adverse Events (AEs)

All AEs (including SAEs) are to be accurately recorded on the Adverse Event page of the subject's electronic CRF from the time the subject 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 subject, actions taken with the study drugs, and the outcome of the AE will also be noted.

7.4 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 anticancer therapy) including the following:

- Any death or SAE experienced by the subject 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 subject 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 subject 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 subject identifiers redacted) will be transmitted 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
- Subject identification (e.g., subject number, initials, sex, age or date of birth)
- Information regarding study drug administration (e.g., 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)

7.5 Sponsor and Investigator Responsibility for Reporting Adverse Events (AEs)

All AEs and SAEs 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.

7.6 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 (eg, 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.

7.7 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.

8. 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 Follow-Up Tumor 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.

8.1 Determination of Sample Size

In view of up to 3 novel investigational drugs being tested in this study, the sponsor has incorporated a Safety Run-in (Part 1) to evaluate safety for each study arm before fully enrolling all subjects required in Stage 1 of Part 2 for each study arm (for details please see Section 5.1.1).

The sample size of Part 2 is determined based on a Simon 2-stage design for each study arm. For Ad-RTS-hIL-12 monotherapy (Arm A) with 13 subjects evaluated in Stage 1 and an additional 20 subjects in Stage 2 if applicable, it will provide 80% power at 1-sided 5% significance level to differentiate 35% targeted proportion of subjects who survive progression-free at week 16 from a proportion of no interest of 15%. If ≥3 of 13 subjects survived progression-free at week 16 in Stage 1, 20 additional subjects will be evaluated in Stage 2.

For the Ad-RTS-hIL-12 + palifosfamide combination (Arm C), with 12 subjects evaluated in Stage 1 and an additional 14 subjects evaluated in Stage 2, it will provide 80% power at 1-sided 5% significance level to differentiate 65% targeted proportion of subjects who survive progression-free at week 16 from a proportion of no interest of 40%. If ≥6 of 12 subjects survived progression-free at week 16 in Stage 1, 14 additional subjects will be evaluated in Stage 2.

8.2 Subject Randomization

Following the safety run-in period in Part 1, subjects will be randomly assigned to Treatment Arms A or C in Part 2 with a 1:1 ratio, adaptively, until reaching the desired number of subjects assigned in each arm. For details please refer to Section 5.1.4.

8.3 Populations for Analysis

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

- Safety Population: All subjects who received at least 1 dose of study drug (INXN-1001, INXN-2001, and/or palifosfamide).

- Efficacy Evaluable Population: All subjects who received at least 1 dose of study drug (INXN-1001, INXN-2001, with or without palifosfamide) and have at least 1 post-screening response evaluation.

8.4 Study Endpoints

8.4.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the 16-week PFS rate, calculated as the number of subjects who had not progressed or died prior to 16 weeks from the date of their first dose, divided by the number of subjects in the study arm. Subjects who neither progressed nor died, but had their last tumor assessment prior to 16 weeks, will not be categorized as progression-free and will not be included in the numerator. Progressive disease (PD) will be determined by mRECIST v1.1 (please refer to Appendix 4).

8.4.2 Secondary Efficacy Endpoints

Tumor-related secondary efficacy endpoints are defined as the following:

- PFS: time from the date of first dose to the date of PD or death (if prior to progression). Subjects without PD or death occurring as of the time of analysis will be censored as of the last date of disease evaluation. PD will be evaluated by mRECIST v1.1

- ORR by mRECIST v1.1: Proportion of subjects achieving a confirmed PR or CR according to mRECIST v1.1 during study. All objective responses (CR or PR) require confirmation by a repeat tumor assessment at least 4 weeks (28 days) after the response is first observed in order for the response to be considered confirmed.

- Duration of response: time from the date of first objective response (PR or CR) by mRECIST v1.1, until the date of PD or 1 year. Duration of response in subjects who have not progressed or died at the time of analysis will be censored as of the date of their last tumor assessment.

- Clinical benefit rate (CBR = proportion of subjects with CR, PR or SD) by mRECIST v1.1

8.4.3 Other Endpoints

Cytokine and breast cancer biomarker (eg, MUC-1) levelswill be explored in serum samples. For study sites participating in additional immunologic assessments, please refer to Appendix 6. INXN-1001 PK analysis will be performed in Cycle 1 for all subjects enrolled the Safety Run-in (Part 1).

8.4.4 Safety Endpoints

The safety endpoints will include AEs, vital signs, ECG, physical examinations, laboratory data, and concomitant medications. All AEs will be coded according to Medical Dictionary

for Regulatory Activities (MedDRA®, version 13.0 or later). Concomitant medications will be coded using the World Health Organization Drug Dictionary, September 2006 or later.

The incidence, onset timing, duration, severity, relationship, and outcome will be listed for each AE. All treatment-emergent AEs (TEAEs) occurring on-study will be analyzed by treatment group and listed in by-subject data listings. Events that are considered related to treatment (possibly related or related) will also be tabulated.

Treatment-emergent AEs will be tabulated overall and by subject. '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 last dose of study drug); 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. Serious TEAEs and related TEAEs will be summarized in a similar fashion. AEs to be presented will include TEAEs, treatment-emergent SAEs, and treatment-emergent related AEs, as well as the severity of the events. Timing relative to study treatment administration will also be presented. Treatment-related SAEs will also be summarized by treatment group and presented on a case-by-case basis in listings and narratives.

Dosing and study drug tolerability will also be assessed.

8.5 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.

8.6 Statistical Analyses

8.6.1 Subject Status

Subject status will be summarized for all randomized subjects by Study Arm. The numbers of subjects in the Efficacy population and Safety population will be summarized, as well as those receiving a non-randomized treatment.

In addition, major protocol violations will be summarized by Study Arm and presented in listings.

8.6.2 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.

8.6.3 Safety Analyses

Safety tables will be presented for all safety parameters including TEAEs, dosing, vital signs, ECGs, and clinical laboratory tests. The Safety Population will be used for these analyses.

AEs will be coded using 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. Deaths, SAEs, and AEs resulting in study discontinuation will be listed.

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

8.6.4 Efficacy Analyses

The Efficacy Evaluable Population will be used for the analyses of efficacy data by study arm.

8.6.4.1 Primary Efficacy Analyses

The primary analysis of the primary endpoint (16-week PFS rate) will be summarized using both a point estimate and its exact binomial 2-sided 95% confidence interval (CI). The 16-week PFS rate will be also estimated using the Kaplan-Meier method. The 95% CI of the PFS rate at 16 weeks will be calculated using the Greenwood formula.

8.6.4.2 Secondary and Other Analyses

PFS is defined per subject as the time (in months) from first dosing date to the date of progression. Subjects who died without a reported prior progression will be considered to have progressed on their date of death. Subjects who did not progress or die will be censored on the date of their last tumor assessment. Duration of response will be computed from the time measurement criteria were met for PR or CR until the date of documented PD or 1 year. Subjects who neither progressed nor died will be censored on the date of last tumor assessment.

INXN-1001 PK parameters to be determined will include, but are 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.

8.7 Immunologic Responses

The trial will assess the cytokine profiles and breast cancer-associated biomarkers (eg, MUC-1) for all subjects.

In addition, participating sites will include additional immunologic assessments as described in Appendix 6. The immunological effects of each treatment cycle on the cellular and humoral immune responses in the peripheral circulation and in tumor biopsy specimens.

Cytokine profiling will be performed by ELISA. Whole blood will be collected for flow cytometry analysis, immune response assay, and/or ELISPOT assays for measurement of the response to breast cancer-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, as well as with serum cytokine levels. In addition, tumor PBx or FNAs will be examined for cytokine expression, presence of injected adenovirus and CTL frequency, Treg and myeloid-derived suppressor cell frequency, and other immunological markers. The change in each measure from baseline and from the preceding biopsy will be correlated with the treatment arm.

8.8 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.

9. STUDY ADMINISTRATION

9.1 Electronic Case Report Forms and Source Documentation

For each subject, eCRFs and corresponding source records will be maintained at each clinical site. The Sponsor or designee will provide the study sites with secure access to and sufficient training on the electronic data capture (EDC) application, to permit site personnel to enter or correct information in the eCRFs for the subjects for whom they are responsible.

eCRFs 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 eCRF 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.

It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's eCRF. Through the EDC application, the investigator must provide formal approval of all subject information in the eCRFs and changes to the eCRFs to endorse the final submitted data for the subjects for whom he/she is responsible. The audit trail entry will show the user's identification information and the date and time of any corrections.

eCRF completion may be delegated to other study personnel; however, such delegation must be documented in writing. 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.

Sponsor or designee will retain the eCRF data and corresponding audit trails. A copy of the final archival eCRF in the form of a compact disk or other electronic media will be placed in the investigator's study file.

9.2 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.

9.3 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, and 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 10.5. 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.

9.4 Duration of the Study

The duration of this study from the time of initiating subject enrollment until the completion of survival follow-up is anticipated to be approximately 34 months, including 24 months for enrollment and the later of 1 year of further follow-up.

Each subject's participation in this study will last approximately 6.5 months, including:

- 28-day screening period.
- 6 cycles (18 weeks) of study treatment.

- Post-Treatment Safety Assessment visit performed 28 days after the last dose of study drug.
- 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 anticancer 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.

9.5 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 2 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 2 additional years and then returned to ZIOPHARM. No records will be destroyed, but will be indefinitely stored.

9.6 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 1 year, as well as satisfying any other local IRB/IEC reporting regulations. Copies of all reports to, and correspondence with, the IRB/IEC must be provided to ZIOPHARM. Further, within 3 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.

9.7 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/IEC 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.

10. INFORMED CONSENT

10.1 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. The Informed Consent 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/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/IEC approval/favorable opinion in advance of use.

10.2 Subject Informed Consent Form

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

11. PROTOCOL APPROVAL PAGE

A Phase II Randomized, Open-Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide-tris in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions

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: _____

[redacted]

13. APPENDICES

13.1 Appendix 1: Eastern Cooperative Oncology Group (ECOG) Performance Status

ECOG PERFORMANCE STATUS[37]

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, eg, 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.

[37] 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.

13.2 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)

13.3 Appendix 3: Regimen for 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, 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.

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.
    - 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 antipyretic 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 tablets 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 antipyretic prophylaxis should be used in all subsequent dose cycles.

13.4 Appendix 4: Modified RECIST Criteria

Progression-free survival (PFS) rate and response to treatment will be determined on the basis of RECIST v.1.1[35] as applied to this trial, taking into account response patterns that have been observed with immunotherapies. In studies with a diversity of immunotherapies in different cancer types, objective responses have been shown to occur after an initial increase in tumor burden characterized as PD by World Health Organization or RECIST criteria.[36]

The 4 observed patterns of response to immunotherapy in subjects with advanced cancer have been:

1. Immediate response - response in baseline lesions-evident by week 12, with no new lesions
2. Durable stable disease - which in some subjects was followed by a slow, steady decline in total tumor burden
3. Response after initial increase in tumor burden
4. Response in the presence of new lesions - a reduction in total tumor burden during or after the appearance of new lesion(s) at time points later than week 12

To capture these response patterns observed with some immunotherapeutic agents, a modification of RECIST v1.1 (mRECIST) will be utilized.

At Baseline:

The disease burden at baseline will be categorized into target and non-target lesions.

A maximum of 5 target lesions (no more than 2 target lesions per organ), representative of all involved organs, may be selected.

Target lesions are lesions that can be accurately measured in at least one dimension and whose minimum lesion size is as follows:

- Non-nodal lesions $\geq$ 10 mm or $\geq$ 2 times the slice thickness/reconstruction interval
- Nodal target lesions (lymph nodes): the short axis must measure $\geq$ 15 mm irrespective of modality/scanner type and slice thickness/interval

Lesions that are/will be injected with INXN-2001 and/or biopsied should not be selected as target lesions.

At Each Subsequent Assessment:

Target lesions are measured at every time point and an overall Sum of Diameters (SOD) will be determined by adding the longest diameters of all non-nodal lesions and short axes (ie, widest dimensions perpendicular to the long axes) of nodal lesions.

Target lesions are assessed as Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD), or Not Evaluable (NE) at every time point based on the SOD.

Target Lesion Assessment	Definition
Complete Response (CR)	CR is declared if all of the following are true for target lesions: • The disappearance of all non-nodal target lesions • Any pathological lymph nodes must have a reduction in short axis to <10 mm.
Partial Response (PR)	PR is declared if there is a decrease of at least 30% in the sum of diameters of target lesions taking as reference the baseline sum of diameters of target lesions.
Progressive Disease (PD)	PD is only declared if all of the following are true for target lesions: • SOD of all target lesions increases at least 20% taking as reference the smallest SOD recorded at or after baseline • Actual SOD increase is ≥5 mm from the smallest SOD recorded at or after baseline
Stable Disease (SD)	SD is declared if target lesion assessment does not meet criteria for PR, PD or CR

Selection and Assessment of Non-Target Lesions:

Non-target lesions are all other lesions, including additional potentially measurable lesions and truly non-measurable lesions, such as lesions that are/will be injected with INXN-2001 and/or biopsied, all bone lesions, ascites, pleural and pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, and brain lesions. Non-target lesions will be assessed qualitatively, and the possible assessments are CR, Non-CR/Non-PD (NN), and PD.

If pleural effusions or ascites (selected as non-target lesions at baseline) are stable in size or minimally enlarging, they will be assessed as NN. New fluid collections or any effusion that significantly worsens will be considered a sign of PD unless cytopathology results are available that support a non-malignant nature.

Non-target lesions should only be assessed as PD if the progression is unequivocal. In the setting of a subject who also has measurable disease, there must be an overall level of substantial worsening in the context of the overall disease burden. A modest increase in size of one or more non-target lesions is not considered sufficient to qualify for unequivocal progression status. In the absence of measurable disease, the change in the overall non-measurable disease burden should be comparable to at least a 20% increase in overall diameter or 73% in approximate overall volume.

New Lesions:

The determination of new lesions should be unequivocal and not be attributable to differences in the scanning technique, change in modality, or findings thought to represent something other than tumor. This is particularly important when the subject's baseline lesions show PR or CR.

New lesions should be reported as equivocal or unequivocal when first noted. New lesions observed at week 12 should not be considered as unequivocally progression; however, they should be re-assessed at subsequent time points. Lesions that were noted as equivocal should be re-assessed as Equivocal, Unequivocal, NN, PD, or Benign on subsequent time points. Lesions that were unequivocal should be re-assessed as Unequivocal, Absent, or NE.

As initial flare-ups have been reported in the context of immunotherapy, new lesions should only be taken as a sign of progression if they are:

- Unequivocally present, and
- Defined as clinically significant.

Clinical significance should relate to the size and location of the new lesion, such as new brain involvement, significant vascular invasion or compression.

Any lesion that does not meet the above criteria should be considered an equivocal new lesion that does not justify in isolation an overall assessment of progression at that time point. Such equivocal new lesions should be re-evaluated at the next time point.

Overall Response Assessments:

Target Lesions	Non-Target Lesions	New Lesions	Time Point Response
CR	**CR**	**No**	**CR**
CR	**NN or NE**	**No**	**PR**
PR	**Non-PD** (including NE)	**No**	**PR**
SD	**Non-PD** (including NE)	**No**	**SD**
PD	**ANY**	**Yes/No**	**PD[a]**
ANY	**PD**	**Yes/No**	**PD[a]**
ANY	**ANY**	**Yes**	**PD[a]**
NE	**Non-PD**	**No**	**NE**

[a] PD at ≤ week 12 requires confirmation at week 16.
NN = Non-CR/Non-PD
NE = Not Evaluable

13.5 Appendix 5: Modification of Diet in Renal Disease (MDRD) Equation for Estimating Glomerular Filtration Rate (eGFR)

The eGFR adjusted for body surface area will be calculated using serum creatinine (SCr) and the 4-variable MDRD equation.[38]

When using isotope dilution mass spectrometry (IDMS)-traceable serum creatinine:

- For creatinine in mg/dL:
  $eGFR\ (mL/min/1.73\ m^2) = 175 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742\ (\text{if female}) \times 1.212\ (\text{if Black})$
- For creatinine in µmol/L:
  $eGFR\ (mL/min/1.73\ m^2) = 30{,}849 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742\ (\text{if female}) \times 1.212\ (\text{if Black})$

When using non-IDMS-traceable serum creatinine:

- For creatinine in mg/dL:
  $eGFR\ (mL/min/1.73\ m^2) = 186 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742\ (\text{if female}) \times 1.212\ (\text{if Black})$
- For creatinine in µmol/L:
  $eGFR\ (mL/min/1.73\ m^2) = 32{,}788 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742\ (\text{if female}) \times 1.212\ (\text{if Black})$

CAUTION REGARDING GFR CALCULATORS

When using GFR calculators, care must be taken to ensure that the correct 4-variable MDRD equation is being applied; some older calculators only use the equation for non-IDMS-traceable creatinine values. The study Medical Monitor MUST be consulted in cases where a local serum creatinine and repeat eGFR determination are performed to determine study eligibility.

Example of an acceptable calculator: The National Kidney Foundation (New York, NY) provides an internet accessible GFR calculator that accommodates IDMS and non-IDMS-traceable creatinine values in either mg/dL or µmol/L. If using this calculator, please select "African American" for all subjects who are Black, regardless of country of origin. The calculator is available on-line at:
http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm

13.6 Appendix 6: Schedule of Study Procedures and Assessments: Additional Immunologic Assessments

[The schedule in this Appendix is ONLY for study sites participating in additional immunologic assessments.]

Part 2 of the Study, ALL Treatment Arms, ALL Cycles
Required procedures from Table 2B are included in this table and are displayed as gray X's

Activity	Screening Day -28 to -1		ALL Cycles Part 2 of the Study, ALL Treatment Arms					Post-Treatment Safety Assessment Visit	Follow-Up Tumor Assessment Visit
	Pre-Registration	Post-Registration	Day 1	Day 2	Day 3	Day 7	Day 15 ±2 days	28 ± 3 days post-last dose study drug	35 ± 7 days after PTSA visit
Clinical Assessments									
Informed Consent	X								
Medical/Cancer History	X								X
Physical Exam	X		X					X	
ECOG PS	X		X					X	
Height	X								
Weight	X		X					X	
Vital Signs	X		X	X	X	X	X	X	
Adverse Events	X	→	→	→	→	→	→	X	
Concomitant Med.	X	→	→	→	→	→	→	→	X
Clinical Laboratory									
Pregnancy test	X		X						
Hematology tests	X		X			X	X	X	
Serum Chemistry tests	X		X			X	X	X	
Urinalysis	X		X				X	X	
ECG	X		X					X	
Subject Registration/ Randomization	X								

Activity	Screening Day -28 to -1		ALL Cycles Part 2 of the Study, ALL Treatment Arms					Post-Treatment Safety Assessment Visit	Follow-Up Tumor Assessment Visit
	Pre-Registration	Post-Registration	Day 1	Day 2	Day 3	Day 7	Day 15 ±2 days	28 ± 3 days post-last dose study drug	35 ± 7 days after PTSA visit
Tumor Response / Pharmacodynamic									
Biopsy / FNA[A]		X				X[A]		X	
PBMC[B] (ie, cellular immune response)		X				X C1 only		X	X
Immune cell evaluation[C] (ie, flow cytometry)		X				X C1, C3 only		X	X
Imaging Studies/Tumor Assessment	X		X C5			X C6			X
Serum cytokine profile & breast CA biomarkers		X	X C5	X C1, C3, C6	X C1, C3, C6	X C1, C3, C6	X C1, C3, C6	X	
Digital photography		X	X C5			X C2, C6		X	X
Study Drug Administration									
Intratumoral INXN-2001			X						
Oral INXN-1001 Arm A cohort 1			←	—	X	—	→		
Verify Adherence to INXN-1001 dosing						X			
Palifosfamide (IV), Arm C			X	X	X				

Footnotes are provided on the following page.

Footnotes:

A. Punch biopsies (PBx) or fine needle aspirate (FNA) samples of tumor(s) and/or tumor-involved draining lymph nodes will be collected for in vivo assessment of immunological activities and other biological effects for all subjects; these samples should be obtained at the time points indicated in the table below. Details of these procedures are described in a separate Laboratory Manual.
NOTE: If a subject is off-study before Cycle 3 then it is imperative that a PBx is obtained at the PTSA visit.

Activity	Screening Day -28 to -1		Cycles 1 and 3 ONLY		Post- Treatment Safety Assessment Visit
	Pre- Registration	Post- Registration	Cycle 1 Day 7±1	Cycle 3 Day 7±1	28±3 days post-INXN-1001 dose
Biopsy / FNA[a]		**X**			
Tumor injected with INXN-2001			**PBx[b]**	**FNA[b]**	**PBx**
Non-Injected Tumor and/or Draining Lymph Node[c]		**PBx[a]**	**PBx[b]**	**FNA[b]**	**PBx**

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

[a] At screening, the PBx should be obtained only AFTER subject registration/randomization has been completed (ie, after approval by sponsor for study participation) and before start of treatment.

[b] PBxs and FNAs on Day 7 should be obtained AFTER administration of INXN-1001.

[c] A pathologic, draining lymph node must be defined as a lesion per mRECIST (see Appendix 4). If a non-injected distal lesion responds to treatment, then a FNA will be obtained to assess the mechanism of the response.

B. Blood sample for cellular immune response (PBMC) analyses to be collected post-registration, Cycle 1 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. **NOTE:** If a subject is off-study before Cycle 3 then it is imperative that a blood sample is obtained at the PTSA visit.

C. Blood sample for analysis of immune cell numbers by flow cytometry should be obtained at screening, Cycle 1 Day 7, 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.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase II Randomized, Open-Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide-tris in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions
Protocol Number:	ATI001-201
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand) Palifosfamide-tris
Date of Protocol:	Original protocol: 17 Aug 2012 Amendment 1: 04 Jan 2013

NOTE TO INVESTIGATORS

Amendment 1 dated 04 Jan 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
Global changes: Editorial	Numerous editorial changes throughout the protocol; additions/revisions made to the list of abbreviations and abbreviations throughout	Clarity and readability
Global change: Primary efficacy endpoint	Throughout the protocol, the primary efficacy endpoint was changed from progression-free rate at week 12 to progression-free rate at week 16. **Tumor response assessments will occur at week 12, 16 and the Follow-up Tumor Assessment week 24**. **Original protocol:** *Primary Objectives*: Assess the efficacy of repeated cycles of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) with INXN-1001 (oral activator ligand) as monotherapy or in combination with palifosfamide-tris as measured by the 12-week progression-free survival (PFS) rate, defined as the proportion of subjects who survive progression-free at week 12 in the study population. **Revised text:** *Primary Objectives:* Assess the efficacy of repeated cycles of intratumoral injections of INXN-2001 (Ad-RTS-hIL-12) with INXN-1001 (oral activator ligand) as monotherapy or in combination with palifosfamide-tris as measured by the **16-week** progression-free survival (PFS) rate, defined as the proportion of subjects who survive progression-free at **week 16** in the study population.	Response patterns that have been observed with immunotherapies in different cancer types include objective responses have been shown to occur after an initial increase in tumor burden characterized by RECIST criteria. Week 16 will better capture these responses.
Global change: Secondary efficacy endpoint	**Remove overall survival as a secondary endpoint of the study** **Added: Assess the PK of INXN-1001 in subjects with recurrent and/or metastatic adenocarcinoma of the breast**	Revise the secondary objectives of the study.
Global change: Response criteria for tumor assessments	**Revised text:** Throughout the protocol, immune-related response criteria (irRC) has been changed to **modified RECIST (mRECIST) criteria**. All treatment arms will utilize mRECIST criteria for tumor response assessments. A new Appendix 4 has been included to provide details for the mRECIST criteria. **Deleted text:** All mentions of immune-related response criteria (irRC) have been deleted. (~~**irRC**~~)	Modified RECIST (mRECIST) criteria are appropriate for assessment of tumor responses in this study.

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Global change: Pharmacokinetic assessment added Part 1 Arm A	**PK assessments were added at the following time points for Arm A Cycles 1 and 2:** **Blood samples for PK analysis of INXN-1001 for Part 1 treatment Arm A CYCLE 1 ONLY should be obtained on the following days and time points:** **Day 1: [6 samples]** Pre-dose (≤30 minutes prior to INXN-1001 dosing), 0.5, 1, 2, 4, 6 hours post-INXN-1001 dosing **Day 2: [1 sample]** Pre-dose (≤ 30 minutes prior to INXN-1001 dosing) **Day 7: [3 samples]** Pre-dose (≤ 30 minutes prior to INXN-1001 dosing), 1-2 hours, 4-6 hours post-INXN-1001 dosing **Day 8: [1 sample]** 24 ± 3 hours post-Day 7 INXN-1001 dose	PK assessment will facilitate and understanding of drug levels and the safety profile for the treatment with study drug
Global change: Study Design Arm A Ad-RTS-hIL-12 Monotherapy has 2 cohorts	**Added text:** **A dose escalation of INXN-1001 will be evaluated with 3 subjects in each cohort of Arm A with safety assessment:** **During Part 1, Arm A will explore the safety of Ad-RTS-hIL-12 monotherapy in 2 cohorts, 3 subjects each. Arm A will enroll cohort 1 at 140 mg INXN-1001 (n=3), and cohort 2 at 160 mg INXN-1001 (n=3). If Ad-RTS-hIL-12 monotherapy is tolerable in 6 subjects (cohorts 1 and 2), then 3 additional subjects will be enrolled during Part 2 for efficacy analysis. All additional subjects enrolled in Part 2 will be at the 160-mg dose of INXN-1001. Futility decisions will be made based on activity observed in subjects receiving INXN-1001 at the 160-mg dose only. If at least one of the 6 subjects (3 + 3) survives progression-free at week 16, then Arm A will be expanded with 7 additional subjects to be randomly assigned to Arm A. These first 13 subjects (6 + 7) on Arm A will undergo tumor assessment for progressive disease (PD) after 16 weeks of treatment. If ≥3 of these 13 subjects survive progression-free at week 16, this stage will be expanded to include 20 additional randomly assigned subjects. If ≥9 of these 33 (13 + 20) subjects survive progression-free at week 16, then this regimen would be considered worthy of further investigation (ie, a confirmatory trial).**	A stepped approach with INXN-1001 dosing will allow for a run-in safety analysis for the study treatment
Global change: Safety assessments of study treatments	**Added and clarified text:** The safety of study treatments will be assessed by the frequency and severity of adverse events (AEs) as determined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 during the Safety Run-in (Part 1) at several stages: • After 3 subjects in the 140 mg cohort of Arm A have completed the first cycle • After 3 subjects in the 160 mg cohort of Arm A have completed the first cycle • After 6 subjects in Arm B have completed the first cycle • After 3 subjects in Arm C have completed the first cycle	Revised timing of safety assessment by stage

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Title Page, Page Headings and Footers	**Added text:** **Amendment 1: 04 January 2013**	Updated Protocol Amendment
Title Page Safety Reporting	**Revised text:** [redacted]	Updated FAX and SAE phone number for safety reporting
List of Abbreviations	**Added text:** **New abbreviations added (eg, PFS, cGMP, CBR, ORR)**	Include additional abbreviations used in the protocol
Section 3 Protocol Synopsis Study Design	**Added text:** **Treatment Arm C: Combination Therapy** Part 1 Arms A and B will complete enrollment of 6 subjects each and monotherapy will be determined to be tolerable before Part 1 Arm C begins. **Part 1** Arm C will evaluate the safety and tolerability of the combination of Ad-RTS-hIL-12 with palifosfamide-tris in 3 subjects **with a 1-week interval between dosing of subjects.** If combination therapy is determined to be tolerable in 3 subjects following one cycle, then this treatment arm will be included in Part 2 of the study.	During Part 1 for Arm C, staggered dosing of subjects for safety observations
Section 3 Protocol Synopsis Exclusion Criteria	**Added text:** **18. Localized infection at site of injectable lesion(s) requiring anti-infective therapy within 2 weeks of the first dose of study drug.** **NOTE: Appropriate testing (eg, punch biopsy [PBx] with bacterial counts) must be performed to rule out infection in cases where the presence of infection is ambiguous or clinical signs of infection are evident.**	Additional exclusion criterion included in protocol
Section 3 Duration of Treatment	**Deleted text:** In the absence of meeting treatment withdrawal criteria, subjects should receive ~~**a minimum of 3 and a maximum of**~~ 6 cycles of study treatment.	Clarify duration of treatment
Section 3 Protocol Synopsis Efficacy Evaluations	**Revised text:** The primary efficacy endpoint is the ~~**12**~~ **16**-week PFS rate defined as the proportion of subjects that remain progression-free at ~~**week 12**~~ **week 16** by ~~**irRC for Arm A (Ad-RTS-hIL-12 monotherapy) and Arm C (Ad-RTS-hIL-12 + palifosfamide combination therapy), and**~~ mRECIST v1.1) for ~~**Arm B (palifosfamide monotherapy)**~~ all Treatment Arms. Survival follow-	Modified RECIST criteria are appropriate for assessment of tumor responses in this study, and week 16 will better

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	up information will be collected for all subjects until 1 year following the completion of enrollment, whichever is later.	capture tumor responses with immunotherapies
Section 3 Protocol Synopsis Experimental Therapy: INXN-1001 + INXN-2001; palifosfamide-tris	**Added text:** • **INXN-1001:** …Each subject will receive INXN-1001 (**cohort 1, 140 mg, n=3; cohort 2 and all other subjects, 160 mg)**. • **INXN-2001:** If only a single lesion is present, then repeat administrations into the same lesion are permitted, **and radiologically-guided techniques may be used** as available.	Indicate that Arm A in Part 1 has 2 cohorts with dose escalation If available, sites may use radiologically-guided techniques to obtain biopsies
Section 3 Table 2A, B Schedule of Assessments	**Added separate Schedule of Assessments for subjects in Arm A cohorts 1 & 2 [contains PK sampling schedule]** **Blood samples for PK analysis of INXN-1001 for Part 1 treatment Arm A CYCLE 1 ONLY should be obtained on the following days and time points:** **Day 1: [6 samples]** Pre-dose (≤30 minutes prior to INXN-1001 dosing), 0.5, 1, 2, 4, 6 hours post-INXN-1001 dosing **Day 2: [1 sample]** Pre-dose (≤ 30 minutes prior to INXN-1001 dosing) **Day 7: [3 samples]** Pre-dose (≤ 30 minutes prior to INXN-1001 dosing), 1-2 hours, 4-6 hours post-INXN-1001 dosing **Day 8: [1 sample]** 24 ± 3 hours post-Day 7 INXN-1001 dose **Added assessments:** **- Vital Signs (blood pressure assessment): Cycle 1 and Cycle 2, Days 3 and 4** **- Imaging Studies/Tumor Assessment: Cycle 6 Day 7 (week 16)** **- Immune cell evaluation by flow cytometry: screening, Cycle 1 and Cycle 3 Day 7, Post-Treatment Safety Assessment visit, Follow-Up Tumor Assessment** **- Breast cancer biomarkers: screening, Cycles 1, 3, 6 Days 2, 3, 7, 15, Post-Treatment safety Assessment visit** **Revised Assessments:** **- PBMC Cycle 1 and Cycle 3 Day 7 only** **- ECG** at screening, **prior to dosing on Day 1 of each cycle, Post-Treatment Safety Assessment visit** **- Digital photography Cycle 2 and Cycle 6 Day 7 (~~Day 21~~)**	Added details for collection of PK samples for subjects in cohorts 1 & 2, Cycle 1 only Additional pharmacodynamic marker sampling added to further explore effects of treatment Modify timing of assessments

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	Edited Table Heading: **Post-Treatment Safety Assessment Visit 28 ± 3 days post-last dose ~~INXN-1001~~ study drug**	Clarified language in table heading
Section 3 Table 2A,B Schedule of Assessments Footnotes	**Added text:** 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. **Subject's blood pressure should 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.** 13. Standard, single 12-lead ECG at screening**, and prior to dosing of INXN-1001 and/or INXN-2001 injection and/or palifosfamide infusion** on Day 1 of each cycle, and at the Post-Treatment Safety Assessment visit. 16. 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 **All imaging should be of diagnostic quality and include IV contrast.** For subjects with measurable lesions, target lesions should be selected and measured as per **modified** RECIST 1.1 guidelines**. (see Appendix 4)** 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)~~ **assessed qualitatively as non-target lesions**. Disease sites are to be imaged throughout the study using the same method(s) used at screening. Specific image acquisition guidelines will be provided. Chest **and** 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. Tumor response assessments will occur at 12 **and 16** weeks (**C4D21 and C6D7**) and the Follow-up Tumor Assessment visit (24 weeks) for all subjects, including those who may have experienced a dose delay. **In the setting of this immunotherapy, a progression observed ≥ week 12 requires confirmation at week 16.** Additional tumor response	Include instruction to monitor blood pressure following INXN-1001 administration Modify timing of ECG to be prior to any study drug administration Remove imaging of pelvis, revised response criteria

<table>
<tr><th>Section in Amended Protocol</th><th>Revised Text/Section</th><th>Rationale for Change</th></tr>
<tr><td></td><td>
assessments may occur at the discretion of the Investigator.

~~**NOTE: In addition to the standard RECIST v1.1 guidelines established to evaluate antitumor responses to chemotherapeutic agents, antitumor response will be assessed using total tumor burden (TTB), where the sum of the products of the two longest diameters (SLD) is used to calculate Total Tumor Burden = SLDindex lesions + SLDnew, for measurable lesions. (Appendix 4)**~~

17. Biopsies (PBx, FNA) will be collected for all subjects according to the table below, and were **removed** for Cycle 6 Day 7 and Follow-up Tumor Assessment.
<table>
<tr><th rowspan="2">Activity</th><th colspan="2">Screening
Day -28 to -1</th><th colspan="2">Cycles 1 and 3 ONLY</th><th>Post-Treatment Safety Assessment Visit</th></tr>
<tr><th>Pre-Registration</th><th>Post-Registration</th><th>Cycle 1
Day 7±1</th><th>Cycle 3
Day 7±1</th><th>28±3 days post-INXN-1001 dose</th></tr>
<tr><td>Biopsy / FNA[a]</td><td></td><td>X[a]</td><td>X[b]</td><td>X[b]</td><td>X</td></tr>
<tr><td>Tumor injected with INXN-2001</td><td></td><td></td><td>PBx[b]</td><td>FNA[b]</td><td>PBx</td></tr>
<tr><td>Non-Injected Tumor and/or Draining Lymph Node</td><td></td><td>PBx[a]</td><td>PBx[b]</td><td>FNA[b]</td><td>PBx</td></tr>
</table>
PBx: Punch biopsy (≥4 mm in diameter); FNA: fine needle aspiration biopsy

[a] At screening, the PBx ~~**and FNAs**~~ should be obtained only AFTER subject registration/randomization has been completed (ie, after approval by sponsor for study participation) and before start of treatment.

[b] PBx's and FNAs on Day 7 should be obtained after ~~**the Day 7**~~ administration of INXN-1001 ~~**for subjects in Arms A and C**~~. **Note: NO PBx will be collected on Cycle 1**
</td><td>Revise biopsy schedule and remove Cycle 6 Day 7and Follow-Up Tumor Assessment visit biopsy</td></tr>
</table>

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	Day 7 for subjects in Cohorts 1&2 of Arm A in Part 1 of the study. [c] **~~PBx is not required at Follow-Up Tumor Assessment visit if ≤30 days since last biopsy.~~ A pathologic, draining lymph node must be defined as a lesion per mRECIST (Appendix 4). If a non-injected distal lesion responds to treatment, then a FNA will be obtained to assess the mechanism of the response.** 19. **Blood sample for analysis of immune cell levels by flow cytometry should be obtained at screening, Cycle 1 Day 7, Cycle 3 Day 7, the Post-Treatment Safety Assessment visit, and Follow-up Tumor Assessment visit. Please refer to the laboratory manual for details regarding sample processing and shipment.** 20. Blood samples for cytokine profile **and breast cancer-associated biomarker levels** should be obtained **at screening**, Day 2 [24 hours (± 4 hours) post-INXN-2001], Day 3, Day 7 and Day 15 of Cycles 1, 3 and 6 and the **Post-Treatment Safety Assessment**. Please refer to the Laboratory Manual for details regarding sample processing and shipment. 21. Digital photograph(s) of all visible injected and non-injected tumor(s), and of any visible local reactions in or around the injected lesion(s). **Photographs are required at screening, Cycle 2 Day 7 or 15, Cycle 4 Day 21, Cycle 6 Day 7, Post-Treatment Safety Assessment and Follow-Up Treatment Assessment visits and ad hoc as the investigator deems necessary.** Details regarding photographic methodology will be provided in a separate manual. 22. Intratumoral INXN-2001 [redacted] injection should be given 3 hours ± 30 minutes after the INXN-1001 dose. **Lesions displaying signs of localized 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 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. 23. 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.**	Add blood sample for flow cytometry assay for immune cell levels Add breast cancer-associated biomarker assessment Revise scheduling for digital photography Clarify lesions displaying signs of localized infection are not to be injected.

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	Subjects should be instructed to maintain good oral hydration on dosing days. 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. 26. Day 1 clinical laboratory assessments may be performed within ~~2~~ **15** days **of the previous cycle through** Day 1 (prior to dosing) ~~the~~ clinic visit.	Indicate that adequate hydration is required during study drug administration.
Section 4.1 Disease Background	**Added text:** The addition of systemic chemotherapy (**palifosfamide**) to immunotherapy (**Ad-RTS-hIL-12**) in patients who are treated for a recurrence or metastasis of breast cancer may decrease the risk of a subsequent local recurrence or distant metastasis. **This combination of therapeutic agents has not yet been studied in human subjects, particularly in this disease; therefore, the safety profile is not yet clearly defined.** The Phase II study proposed in this protocol (ATI001-201) will **therefore** examine the safety, anti-tumor activity, and immunological effects of intratumoral injections of INXN-2001 + orally administered INXN-1001 (Ad-RTS-hIL-12 monotherapy) alone and in combination with palifosfamide in subjects with advanced breast cancer.	Clarify that a primary objective of this study is to determine the safety of these two agents which have not previously been tested in metastatic breast cancer
Section 4.2.3 INXN-1001 (Activator Ligand)	INXN-1001 is an orally active small molecule diacylhydrazine and is fully active at the RTS[redacted] receptor. INXN-1001 drug product is formulated ~~**(referred to as F-22 formulation)**~~ as a semi-solid containing INXN-1001 as a dry powder and excipients.	Revise language around formulation to simplify
Section 4.3.1 IL-12 Immunotherapy	**Added text:** Interleukin (IL)-12 is a potent immunostimulatory cytokine which activates and recruits dendritic cells (DC) that 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, **augmentation of antigen-specific anti-tumor responses**, and the production of IFN-γ and TNF-α.[11-14] **IL-12 also ~~has anti-angiogenic activity~~ stimulates neutrophil and macrophage production of superoxides and nitric oxide, and induces the production of anti-angiogenic factors by tumor cells. IL-12 has been shown to have anti-tumor activity in several animal models.** **It is therefore important to achieve the immunological benefits that IL-12 provides while avoiding the toxicity elicited by systemic delivery. IT delivery of Ad-RTS-hIL-12**	Clarify the rationale for IL-12 immunotherapy

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	accomplishes precisely this objective by allowing adjustment of IL-12 gene transription by varying the dose of INXN-1001. The vector is localized in the tumor and the gene is produced locally in response to predetermined doses of the oral ligand. In this scenario the dose can be lowered if any toxicities are observed.	
Section 4.3.2 Palifosfamide-tris (Palifosfamide)	Palifosfamide is a novel bi-functional DNA alkylating agent **that has activity in multiple tumors by evading typical resistance ~~which is not metabolized by aldehyde dehydrogenase (ALDH), nor is a substrate for multi-drug resistance~~** pathways. **~~Therefore, it is likely to have activity, and be effective, in multiple tumors using typical resistance pathways~~. Palifosfamide is in the same class as bendamustine, cyclophosphamide, and ifosfamide.**	Revise mechanism of action statement for palifosfamide
Section 4.4.1 INXN-1001 and INXN-2001	[redacted]	Revise language around formulation to simplify

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	[redacted]	
Section 4.5 Rationale for Study Design and Dose Selection	**Added text:** The objectives of this Phase II clinical trial are to assess the safety and tolerability, PFS rate at 16 weeks, ORR, and immunological and biological effects of IT injections of INXN-2001 + INXN-1001 as monotherapy or in combination with palifosfamide. To provide an opportunity to monitor the safety of Ad-RTS-hIL-12 monotherapy, palifosfamide monotherapy, and Ad-RTS-hIL-12 + palifosfamide in subjects with breast cancer **(an indication where neither therapy has been tested)**, a safety run-in will occur, with evaluation following one cycle of study drug. **In addition, the study will commence with Ad-RTS-hIL-12 monotherapy using a 140 mg dose of INXN-1001 in 3 subjects, which will then increase to 160 mg in a second cohort of 3 subjects, following a safety assessment. This 2-cohort dose escalation is being employed to allow for safety monitoring in this population of subjects with breast cancer.** Safety **(Part 1)** and ~~initial efficacy~~ will determine commencement of ~~Part 2 with~~ randomization.	Clarify modification to study design to include a 2-cohort safety run-in with 2 doses of INXN-1001
Section 5.1 Objectives	**Added text:** • **Assess the PK of INXN-1001 in subjects with recurrent and/or metastatic adenocarcinoma of the breast** • [redacted]	[redacted]

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 5.1.3 Safety Review Committee	**Added 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 after 1 cycle during the initial Ad-RTS-IL-12 and palifosfamide monotherapy arms, and the Ad-RTS-hIL-12 + palifosfamide combination therapy arm as described below and in Section 5.1.5,~~ **during the Safety Run-in (Part 1) at several stages:** • **After 3 subjects in the 140 mg cohort of Arm A have completed the first cycle** • **After 3 subjects in the 160 mg cohort of Arm A have completed the first cycle** • **After 6 subjects in Arm B have completed the first cycle** • **After 3 subjects in Arm C have completed the first cycle** and at any other time as needed following random assignment in Part 2. **The safety run-in of Arm C (n=3) will occur with a 1-week interval between dosing of subjects and an SRC will convene after all subjects have received 1 cycle of therapy.** **Deleted text:** Following a treatment arm review, the SRC may recommend proceeding with enrolling additional subjects in the current treatment arm or not enrolling any additional subjects. **If a 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 or palifosfamide on a case by case basis. Any subject who experiences a Grade 4 toxicity related to study treatment will be withdrawn from study.**	During Part 1 for Arm C, staggered dosing of subjects for safety observations
Section 5.1.4 ~~Randomization~~ **Enrollment Procedure**	**Revised text:** During **Part 1**, **3** eligible subjects will enroll ~~initially~~ **into Arm A cohort 1, followed by 3 subjects in Arm B, followed by enrollment of the next 6 subjects** in parallel under alternating assignment, followed by ~~enrollment~~ **3 subjects in Arm C, as per the following pattern: AAA, BBB, ABABAB, CCC** **This pattern may be subject to change based on Sponsor discretion.** During **Part 2**, eligible subjects will be ~~centrally~~ randomly assigned ~~using an interactive response system established~~ by the Sponsor or its representative:	Clarify enrollment process into treatment arms during Part 1 of the study

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 5.1.5 Treatment Arm Expansion Procedure	**Deleted text:** ~~**Three treatment arms are planned for the safety run-in part of the study as described in Table 2. Palifosfamide monotherapy Arm B will complete enrollment with 6 subjects in Part 1 of the study and will not proceed to Part 2.**~~ **Added text:** **Based on a review of available safety and clinical response data, the SRC may identify a single treatment arm (Arm A and/or Arm C) to be expanded to better define the safety, tolerability and activity of the study treatment. Expansion decisions will be made after evaluation of tumor response at week 16. Should a subject discontinue study treatment before receiving 6 cycles for reasons other than progression, another subject may be added to the treatment arm to provide a total of 3 or 6 subjects for full analysis. Response evaluation and criteria for treatment arm expansion or futility are described in Section 9 Statistical Methods.** **Deleted text:** ~~**Two separate analyses will be performed: a safety analysis after cycle 1 (all 3 arms) and an efficacy analysis after week 12 (any open treatment arms). Initially, 6 subjects will be enrolled into the Ad-RTS-hIL-12 monotherapy arm. After the last subject has completed the first cycle of treatment, a meeting of the SRC will be convened to review the overall occurrence of adverse events and relevant laboratory data in the treatment arm. Following this review, the SRC will advise on subject enrollment. The same process will be followed concurrently for the palifosfamide monotherapy (n=6) followed by Ad-RTS-hIL-12 + palifosfamide combination therapy (n=3) safety run-in arms.**~~ ~~**Based on a review of available safety and clinical response data, the SRC may identify a single treatment arm (Arm A and/or Arm C) to be expanded to better define the safety, tolerabilitycycles for reasons other than toxicity, another subject may be added to the treatment arm to provide a total of 3 or 6 subjects for full analysis. Response evaluation and criteria for treatment arm expansion or futility are described in Section 10 Statistical Methods.**~~	Clarify treatment arm expansion and replacement of subjects

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 6.2 Exclusion Criteria	**Added text:** 18. **Localized infection at site of injectable lesion(s) requiring anti-infective therapy within 2 weeks of the first dose of study drug.** **NOTE: Appropriate testing (eg, punch biopsy with bacterial counts) must be performed to rule out infection in cases where the presence of infection is ambiguous or clinical signs of infection are evident.**	Additional exclusion criterion included in protocol
Section 6.4 Replacement of Subjects	**Revised Text:** ~~**During Part 1, 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 treatment arm of subjects completes Cycle 1 safety evaluations. In addition, S**~~should a subject discontinue study treatment before receiving ~~**4**~~ **6** cycles for reasons other than progression, another subject may be added to that study arm for full analysis.	Clarification and correction of typo
Section 7.2.3 Treatment Regimen	**Added text:** 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 of approximately [redacted] 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 an alternate lesion or a draining, **pathologic** lymph node of a previously accessible lesion. **A pathologic lymph node must be defined as a lesion per mRECIST (Appendix 4).** 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 on and between dosing days. Blood pressure should be monitored regularly.**	Indicate that adequate hydration and blood pressure monitoring are required during study drug administration.
Section 7.2.4.3 Preparation and Administration of INXN-2001	**Added text:** 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 approximately [redacted]. If another tumor is not available, then the remaining volume should be injected into a draining, **pathologic** lymph node of the injected tumor. At any cycle, if no accessible lesion for INXN-2001 injection is present (eg, due to complete resolution), then INXN-2001 should be injected into a draining, pathologic lymph node of a previously accessible lesion. **A pathologic lymph node must be defined as a lesion per mRECIST (Appendix 4).** **Note: In the absence of the availability of radiographically-guided biopsies,** 1 accessible lesion will not be injected since that lesion will be used to evaluate the systemic effect of INXN-	Clarify definition of draining, pathologic lymph node

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	2001.	
Section 7.2.5 Retreatment Criteria, Dose Delays and Modifications	**Added text:** Treatment delays for recovery to acceptable levels are allowed at weekly intervals up to a maximum of ~~**3 weeks**~~ **14 days**. In the event that a treatment-related dose delay is necessary, then the next cycle of study treatment will be administered at 80% of the dose (Table 3). If an AE is related to one study drug and not the other (in combination Treatment Arm C) then dose modification/delay occurs for both study drugs (**palifosfamide and INXN-1001**).	Clarify time for treatment delays, and with which drugs
Section 7.2.7 Prophylactic Antipyretic and/or Analgesic Administration	**Added text:** 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 and **up to Day 4 of each cycle**.	Allow for administration of antipyretics and/or analgesics at appropriate times
Section 7.4.2.5 Vital Signs	**Added text:** 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 3 and Day 4 for Cycle 1 and Cycle 2.**	Include instruction to monitor blood pressure following INXN-1001 administration.
Section 7.4.2.11 Electrocardiograms (ECGs)	**Revised text:** A standard, single, 12-lead ECG for local safety assessment will be ~~**done**~~ performed at screening, ~~**2½ to 3 hours after INXN-1001 dosing (and prior to palifosfamide infusion and INXN-2001 injection)**~~ prior study drug administration 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.	Clarify that ECGs are to be performed prior to study drug administration on Day 1 of each cycle.
Section 7.4.2.13 Immune Response Analyses	**Deleted text:** 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 biomarkers ~~**(eg, CEA, CA15-3, CA27.29 or other breast cancer specific biomarkers)**~~ or 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).	Delete specific markers to be analyzed
Section 7.4.2.14 INXN-1001 Pharmacokinetic Assessment	**Added text:** **INXN-1001 PK assessment will occur for subjects enrolled in Part 1 Arm A (Ad-RTS-hIL-12 monotherapy) during Cycle 1 of study treatment. Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day 8 as defined in Table 2A, Schedule of Assessments.**	Specify the sampling for PK during Cycle 1

Section in Amended Protocol	Revised Text/Section	Rationale for Change
Section 7.4.3 Efficacy Assessments	**Revised and added text:** 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** **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 each subject, disease sites are to be assessed throughout the study using the same method(s) of assessment used at screening. Chest and abdomen imaging are 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.** ~~**In addition 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.[38] Total tumor burden is allowed to progress up to 25% from baseline, and new lesions arising do not define progression unless the total burden of target and new lesions summed is increased by more than 25%. Please refer to Appendix 4 for a further description of irRC.**~~ ~~**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 **m**RECIST v1.1 guidelines. **For this trial, disease progression will require at least one of the following:** • **a 20% increase, in addition to an absolute minimum increase of 5 mm in the Sum of Diameter of the target lesions,** • **an unequivocal progression of the non-target lesions,** • **a clinically significant new lesion.** **In the setting of this immunotherapy, a progression observed ≤ week 12 requires confirmation at week 16. (see Appendix 4 for further details)** Subjects without objective evidence of disease progression at the Follow-Up Tumor Assessment visit should continue to	Clarify imaging guidance and align with Schedule of Assessments Redundant with other language in this section

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	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.	
Section 7.4.4.1 Transgene Function	**Added text:** **If a non-injected distal lesion responds to treatment, then a FNA will be obtained to assess the presence of the adenoviral vector and mechanism of the response.**	Indicate the collection of a FNA in the event a non-injected distal lesion responds to determine the mechanism of response
Section 7.4.4.2 Immunological Activities	**Deleted text:** 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 ~~NK~~ CD56 cells, will be evaluated as well as immune suppressor elements, such as T-regulatory cells (Tregs). Biopsy specimens will also be evaluated for markers of immune activation. Blood will be drawn for assessing cell-mediated immunity, especially frequency of cytotoxic T lymphocytes (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.**~~ PBMCs incubated with a pool of breast cancer antigen-derived peptides will be assayed by ~~**IFN γ**~~ ELISPOT and/or flow cytometric intracellular staining for ~~**IFN γ and other**~~ relevant cytokines to enumerate the anti-breast cancer CTLs.	Delete specific markers to be analyzed
[redacted]	[redacted]	[redacted]
Section 9.4.2 Secondary Efficacy Endpoints	**Deleted text:** ~~**In addition 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 with irRC over time. Please refer to Appendix 4 for a**~~	Revised tumor response criteria

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	~~description of irRC.~~	
Section 9.4.3 Other Efficacy Endpoints	**Deleted and added text:** Pharmacodynamic tumor markers may be explored (eg, ~~circulating tumor cells,~~ cytotoxic T lymphocytes, other breast cancer-associated biomarkers ~~such as CA15-3, CA27.29, carcinoembryonic antigen~~) in serum or tumor tissue samples. **INXN-1001 PK analysis will be performed in Cycle 1 for subjects enrolled in Arm A. during the Safety Run-in (Part 1).**	Delete specific markers to be analyzed
Section 9.6.4.2 Secondary and Other Efficacy Analyses	**Deleted text:** PFS is defined per subject as the time (in months) from first dosing date to the date of progression. Subjects who died without a reported prior progression will be considered to have progressed on their date of death. Subjects who did not progress or die will be censored on the date of their last tumor assessment. ~~The overall survival is defined as the time (in months) from first dosing date until the date of death. For those subjects who did not die, survival duration will be censored at the last date the subject was known to be alive.~~ Duration of response will be computed from the time measurement criteria were met for PR or CR until the date of documented PD or ~~death~~ **1 year**. Subjects who neither progressed nor died will be censored on the date of last tumor assessment. **Added text:** **INXN-1001 PK parameters to be determined will include, but are 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.**	Delete overall survival as an endpoint
Section 9.7 Immunological Responses	**Revised text:** 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. Cytokine profiling will be performed by multiplex analysis. Whole blood will be collected for flow cytometry analysis, **immune response assay** and/or ELISPOT assays ~~to test anti-tumor T lymphocyte responses against adenovirus and/or the RTS™ components as well as~~ against breast cancer-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 ~~IL-12~~ **cytokine** expression,	Delete specific markers to be analyzed, add assay methodology

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	presence of injected adenovirus and CTL frequency, Treg and myeloid-derived suppressor cell frequency, and other immunological markers. The change in each measure from baseline and from the preceding biopsy will be correlated with the treatment arm.	
Section 14.4 Appendix 4	**Deleted: ~~Appendix 4 Immune Related Response Criteria~~** **Added: Appendix 4 Modified RECIST Criteria:** **Progression free survival (PFS) rate and response to treatment will be determined on the basis of RECIST 1.1[39] as applied to this trial, taking into account response patterns that have been observed with immunotherapies. In studies with a diversity of immunotherapies in different cancer types, objective responses have been shown to occur after an initial increase in tumor burden characterized as progressive disease by World Health Organization or RECIST criteria.[40]** **The 4 observed patterns of response to immunotherapy in patients with advanced cancer have been:** **1. Immediate response - response in baseline lesions-evident by week 12, with no new lesions** **2. Durable stable disease - which in some patients was followed by a slow, steady decline in total tumor burden** **3. Response after initial increase in tumor burden** **4. Response in the presence of new lesions - a reduction in total tumor burden during or after the appearance of new lesion(s) at time points later than week 12** **To capture these response patterns observed with some immunotherapeutic agents, a modification of RECIST v1.1 will be utilized.** **At Baseline:** **The disease burden at baseline will be categorized into target and non-target lesions.** **A maximum of 2 target lesions per organ and 5 target lesions in total, representative of all involved organs, may be selected.** **Target lesions are lesions that can be accurately measured in at least one dimension and whose minimum lesion size is as follows:**	Response patterns that have been observed with immunotherapies in different cancer types include objective responses have been shown to occur after an initial increase in tumor burden. This study will capture these responses using modified RECIST criteria, which are described in detail in this new Appendix.

<table>
<tr><th>Section in Amended Protocol</th><th>Revised Text/Section</th><th>Rationale for Change</th></tr>
<tr><td></td><td>

• Non-nodal lesions ≥ 10 mm or ≥ 2 times the slice thickness/reconstruction interval
• Nodal target lesions (lymph nodes): the short axis must measure ≥ 15 mm irrespective of modality/scanner type and slice thickness/interval

Lesions that are/will be injected with INXN-2001 and/or biopsied should not be selected as target lesions.

At each subsequent Assessment:
Target lesions are measured at every timepoint and a single Sum of Diameters (SOD) will be determined by adding the longest diameters of all non-nodal lesions and short axes (ie, widest dimensions perpendicular to the long axes) of nodal lesions. Target lesions are assessed as Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD) or Not Evaluable (NE) at every time point based on the SOD.

<table>
<tr><th>Target Lesion Assessment</th><th>Definition</th></tr>
<tr><td>Complete Response (CR)</td><td>CR is declared if all of the following are true for target lesions:
• The disappearance of all non-nodal target lesions
• Any pathological lymph nodes must have a reduction in short axis to < 10 mm.</td></tr>
<tr><td>Partial Response (PR)</td><td>PR is declared if there is a decrease of at least 30% in the sum of diameters of target lesions taking as reference the baseline sum of diameters of target lesions.</td></tr>
<tr><td>Progressive Disease (PD)</td><td>PD is only declared if all of the following are true for target lesions:
• SOD of all target lesions increases at least 20% taking as reference the smallest SOD recorded at or after baseline
• Actual SOD increase is ≥ 5 mm from the smallest SOD recorded at or after baseline</td></tr>
<tr><td>Stable Disease (SD)</td><td>SD is declared if target lesion assessment does not meet criteria for PR, PD or CR</td></tr>
</table>
</td><td></td></tr>
</table>

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	Selection and Assessment of Non-Target Lesions **Non-target lesions are all other lesions, including additional potentially measurable lesions and truly non-measurable lesions such as lesions that are/will be injected with INXN-2001 and/or biopsied, all bone lesions, ascites, pleural and pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, and brain lesions. Non-target lesions will be assessed qualitatively, and the possible assessments are CR, Non-CR/Non-PD (NN), and PD.** **Non-target lesions should only be assessed PD if the progression is unequivocal. In the setting of a patient who also has measurable disease, there must be an overall level of substantial worsening in the context of the overall disease burden. A modest increase in size of one or more non-target lesions is not considered sufficient to qualify for unequivocal progression status. In the absence of measurable disease the change in the overall non-measurable disease burden should be comparable to at least a 20% increase in overall diameter or 73% in approximate overall volume.** **New Lesions** **The determination of new lesions should be unequivocal and not be attributable to differences in the scanning technique, change in modality or findings thought to represent something other than tumor. This is particularly important when the patient's baseline lesions show partial or complete response.** **New lesions should be reported as equivocal or unequivocal when first noted. New lesions observed at week 12 should not be considered unequivocally progression; however, they should be re-assessed at subsequent time points. Lesions that were noted as equivocal should be re-assessed as Equivocal, Unequivocal, NN, PD, or Benign on subsequent timepoints; Lesions that were unequivocal should be re-assessed as Unequivocal, Absent or NE.** **As initial flare ups have been reported in the context of immunotherapy, new lesions should only be taken as a sign of progression if they are:** • **Unequivocally present, and**	

Section in Amended Protocol	Revised Text/Section	Rationale for Change
	• **Defined as clinically significant.** **Clinical significance should relate to the size and location of the new lesion, such as new brain involvement, significant vascular invasion or compression.** **Any lesion that does not meet the above criteria should be considered an equivocal new lesion that does not justify in isolation an overall assessment of progression at that time point. Such equivocal new lesions should be re-evaluated at the next time point.**	

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase II Randomized, Open-Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions
Protocol Number:	ATI001-201
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand) Palifosfamide
Date of Protocol:	Amendment 2: 17 June 2013 Amendment 1: 04 Jan 2013 Original protocol: 17 Aug 2012

NOTE TO INVESTIGATORS

Amendment 2 dated 17 June 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 2: 17 June 2013	Updated Protocol Amendment
Abbreviations and Definitions of Terms	Several new abbreviations were included: eg, MCV, RXR.	Clarify abbreviations
Global	• Study Design changes include: o Arm A Cohorts 1 and 2 have been removed in Part 1; design for this Amendment is a single Arm A Ad-RTS-hIL-12 monotherapy in 6 subjects in Part 1 at 140 mg oral INXN-1001 administered daily x 7 days + a single intratumoral injection of INXN-2001 [redacted] with a 21-day cycle. o Arm B (Palifosfamide monotherapy) has been removed from the study: Arm B of this study, palifosfamide as a monotherapy, was initially included to enhance the safety run in portion of the study and first time use in this therapeutic indication. In early 2013, ZIOPHARM completed (under IND 61792) a study entitled, "A Phase I Multicenter, Open-label Study to Assess the Drug Metabolism and Pharmacokinetics (DMPK) of Palifosfamide-tris and Bioequivalence of Aseptically-processed and Terminally-sterilized Drug Product in Subjects with Advanced Malignancies". As part of this study, 3 subjects with advanced breast cancer were enrolled and received 150 mg/m^2 palifosfamide. There were no serious adverse events experienced by these subjects which demonstrate that palifosfamide as a monotherapy in breast cancer subjects appears to be safe. It should also be noted that the dose of palifosfamide to be used in this combination study is lower (120 mg/m^2). As a result, Arm B of this study has been removed.	Clarity and accuracy, correct redundancy

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	○ The dose of palifosfamide has been changed from ~~130~~ to 120 mg/m^2 ○ Updated Study Design Figure 1 and Table 1 ○ Many subsections of the Protocol Synopsis were condensed for clarity and simplicity ○ Pharmacokinetic assessments will occur in Part 1 Cycle 1 only. • All references to palifosfamide ~~-tris~~ have been changed to palifosfamide • ~~Nonreplicative~~ has been replaced by replication-incompetent for descriptions of the adenoviral vector INXN-2001 • Some nonclinical data also available in the current Investigator's Brochure were deleted • Appendix 6 was added with a separate schedule of assessments for participating sites with subjects enrolled in the additional immunological assessment portion of this study • [redacted] • Corrections in grammar, style	Based on most effective dose in the animal study. See Appendix 1.
Section 3 Protocol Synopsis Study Design	**Revised Table 1 and Figure 1**	Update study design and revise descriptions of the study Phases
Section 3 Protocol Synopsis Inclusion Criteria **Section 6.1**	2. Histologically or cytologically confirmed adenocarcinoma of the breast, either locally recurrent or metastatic disease with injectable lesions, for which no proven ~~effective~~ curative therapy exists. Locally recurrent disease must not be amenable to surgical resection or radiation with curative intent.	Revise text for clarity

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Inclusion Criteria		
Section 3 Protocol Synopsis Exclusion Criteria **Section 6.2** Exclusion Criteria	3. Prior therapies discontinuation periods: a) Radiation within 3 weeks of enrollment b) Chemotherapy within 4 weeks of enrollment c) Nitrosoureas within 6 weeks of enrollment d) Biologic therapy and/or immunomodulatory therapy (eg, granulocyte colony stimulating factor [GCSF]/granulocyte macrophage colony stimulating factor [GM-CSF], interferons or interleukins, growth hormone, intravenous immunoglobulin [IVIG], retinoic acid), checkpoint inhibitors (eg, ipilimumab, anti-PD-1 or anti-PDL-1 antibodies) within 6 weeks of enrollment e) 4. No washout period is required for endocrine therapy 7. Immunosuppressive therapy: a) Systemic immunosuppressive drugs including corticosteroids (prednisone equivalent >10 mg/day, http://www.medcalc.com/steroid.html) within 6 weeks b) Immune suppression/requiring immunosuppressive drugs, including subjects with organ allografts 10.	Updated criteria to reflect currently available checkpoint inhibitors, prior therapy washout periods
Section 3 Protocol Synopsis Experimental Therapy Dose and Schedule	**Revised text:** INXN-1001 will be supplied by the Sponsor as a capsule for oral administration. INXN-2001 will be supplied by the Sponsor as a sterile suspension for injection. Palifosfamide will be supplied by the Sponsor as a powder in single-use glass vials.	Update language to reflect study design and simplify the language regarding the fed state for study subjects

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Dose & Schedule (21-day cycle): • **INXN-1001:** Subjects will be directed to take the INXN-1001 oral dose in a fed state (within approximately 30 minutes following a normal meal), once daily at the same time each day (±1 hour), beginning on Day 1 of each cycle. Each subject will receive INXN-1001 (140 mg daily for 7 days). • **INXN-2001**: INXN-2001 [redacted] IT injection administered 3 hours (± 30 minutes) after INXN-1001 dosing on Day 1 of each cycle. If >1 injectable lesion is present, INXN-2001 will be injected into a different lesion at each cycle, and if the number of lesions is limited, the injections will be administered in sequential rotation or at investigator's discretion. If only a single lesion is present, then repeat administrations into the same lesion are permitted, and radiologically-guided techniques may be used as available. **Palifosfamide:** Palifosfamide (120 mg/m^2/day) will be administered by intravenous (IV) infusion over approximately 30 minutes on Days 1-3 of each cycle.	Revise dose of palifosfamide
Section 3 Protocol Synopsis Pharmacodynamic Evaluations	**Revised text:** Biological markers of response will be assessed and will include examinations of cytokine and breast cancer-associated biomarker (eg, MUC-1) levels. For certain participating sites, additional immunological analyses will be performed. Please refer to Appendix 6.	Modified to simplify the protocol to reflect all subjects will have biologic markers of response (cytokines, breast ca biomarker) assessed Additional immunological analyses will be performed at participating sites and are described in a new Appendix 6
Section 3 Protocol Synopsis Pharmacokinetics	**Revised text:** INXN-1001 PK will be assessed for subjects in in Part 1 Cycle 1 only.	Clarify PK assessments will be in Part 1 Cycle 1 only
Section 3 Table 2A, B	**Updates were made to both of the Schedule of Assessments Tables:**	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Schedule of Assessments and Footnotes	○ **Table 2A Schedule of Assessments included are for Part 1 Cycle 1 ONLY** ○ **Table 2B Schedule of Assessments included are for All Cycles except for Part 1 Cycle 1** • Footnote numbering has changed **Footnotes**: 6. Vital signs include blood pressure, pulse, temperature, and respirations. On Days 1, 2, and 3 of each cycle, vital signs will be recorded prior to study drug dosing. Blood pressure should be monitored closely, with hydration as needed, to prevent hypotension after administration of INXN-1001. [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 case report form (CRF); AEs/SAEs that occur prior to informed consent should be added to the medical history 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 anticancer therapy. 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	Clarify Study visits and Assessments; simplify schedule of assessments Add instructions for hydration Reduce redundancy

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	thromboplastin time) and INR (international normalized ratio) will also be evaluated. 14. 15. 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 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 All imaging should be of diagnostic quality and include IV contrast. PET imaging is an acceptable alternative modality provided the CT component is of diagnostic quality as per RECIST 1.1 guidelines. For subjects with measurable lesions, target lesions should be selected and measured as per mRECIST v1.1 guidelines. (Appendix 4) Lesions that will be/are injected with INXN-2001 and/or biopsied should not be selected as target lesions, but should be assessed qualitatively as non-target lesions. Disease sites are to be imaged throughout the study using the same method(s) used at screening. Chest and abdomen imaging are 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. Tumor response assessments will occur at 12 weeks and 16 weeks (C5D1 and C6D7), and the Follow-up Tumor Assessment visit (24 weeks) for all subjects, including those who may have experienced a dose delay. In the setting of this immunotherapy, a progression observed at week 12 requires confirmation at week 16. Additional tumor response assessments may occur at the discretion of the investigator. 16. A sample(s) for cytokine profiling, breast cancer-associated biomarker levels (eg, MUC-1), and also INXN-1001 levels. Please refer to the Laboratory Manual for details regarding sample processing and shipment.	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	○ Screening (post-registration) ○ Cycle 1 - Days 2, 3, 7, and 15 ○ Cycle 3 - Days 2, 3, 7, and 15 ○ Cycle 5 - Day 1 (to coincide with the tumor assessment) ○ Cycle 6 - Days 2, 3, 7, and 15 ○ Post-treatment Safety Assessment 16. Punch biopsies are now part of the "additional Immunologic Assessments" as part of Appendix 6 for participating sites. 18. Intratumoral INXN-2001 [redacted] injection should be administered 3 hours ± 30 minutes after the INXN-1001 dose. Lesions displaying signs of localized infection should not be injected. Subjects must be instructed to maintain good oral hydration on and between dosing days. [redacted] 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. 19. The subjects will be dispensed INXN-1001 for self-administration after Day 1. The first INXN-1001 dose will be given at the clinical site in a fed state (within approximately 30 minutes after the start of a normal meal). The remaining 6 doses can be self-administered at the same time as on Day 1 (±1 hour) in the fed state. Study sites must verify adherence with INXN-1001 dosing. Subjects should return bottles of INXN-1001 to the clinical site to determine extent of subject adherence to self-administration preferably on Day 7. 22. Post-Treatment Safety Assessment visit will be performed 28 ± 3 days after	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	the last dose of study drug or prior to receiving another anticancer therapy. 23. A Follow-Up Tumor Assessment visit will be performed 35 ± 7 days after the Post-Treatment Safety Assessment visit. 24. 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 4.3.2 Palifosfamide	**Revised text:** Palifosfamide is currently being investigated in a Phase III clinical trial. The MATISSE trial is evaluating palifosfamide (130 mg/m^2 × 3 q21 days) in combination with carboplatin and etoposide as a treatment for subjects with extensive-stage small cell lung cancer.	Updated with recent trial experience for palifosfamide
Section 4.3.3 Nonclinical Efficacy Studies	Nonclinical studies are described in the Investigator's Brochure and were deleted.	Simplify the protocol
Section 4.4 Summary of Prior Clinical Experience and Safety	**Section 4.4.1** This section was updated per the most recent Investigator's Brochure with clinical safety experience in 15 subjects in the ATI001-101 trial in subjects with metastatic melanoma. **Section 4.4.4** In addition, the palifosfamide safety experience was updated following the blinding safety data cut for the Phase III trial in soft-tissue sarcoma with n=437 subjects.	Updated with recent trial experience/results for palifosfamide
Section 4.5 Rationale for Study Design and Dose Selection	The objectives of this Phase II clinical trial are to assess the safety and tolerability, PFS rate at 16 weeks, ORR, and immunological and biological effects of IT injections of INXN-2001 + INXN-1001 as monotherapy or in combination with palifosfamide. To provide an opportunity to monitor the safety of Ad-RTS-hIL-12 monotherapy and Ad-RTS-hIL-12 + palifosfamide (combination therapy) in subjects with breast cancer, a safety run-in will occur, with evaluation following one cycle of study drug. The study will commence with Ad-RTS-hIL-12 monotherapy using a 140-mg dose of INXN-1001 administered daily Days 1-7 in 6 subjects (21-day cycle). Following a safety assessment, combination therapy of Ad-RTS-hIL-12 + palifosfamide (120 mg/m^2, Days 1, 2, 3 of a 21-day cycle, n=3) will commence. Safety in Part 1 of the study will determine commencement of randomization for	Clarify Study Design as 2 Arms, 2 Parts Reduce redundant text

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Part 2.	
Section 6.1 Study Objectives	**Revised text:** • Evaluate pharmacodynamic tumor markers (eg, breast cancer-associated biomarkers such as MUC-1)	Align with current study objectives, cytokines and breast cancer biomarkers will be assessed for all subjects
Section 5.1.1.1 Overall Study Design	**Deleted text:**	Deleted to improve clarity
Section 5.1.2 Treatment Parameters and Duration	**Revised text**: Subjects will receive IT injections of INXN-2001 [redacted] per injection) on Day 1 of each cycle, will also receive oral doses of INXN-1001 (140 mg daily) for 7 days. Subjects enrolled in Arm C combination therapy will also receive palifosfamide (120 mg/m^2/day) by IV infusion over approximately 30 minutes on Days 1, 2, 3 of each cycle.	Update study design and revise descriptions of the study Phases; revise dosing regimen
Section 5.1.3 Safety Review Committee (SRC)	**Revised text:** An SRC comprised of the Medical Monitor, enrolling PIs, and Sponsor representatives will hold periodic teleconferences to evaluate the safety and treatment status of all subjects available during the Safety Run-in (Part 1) at several stages: • after 6 subjects Arm A have completed the first cycle, • after 3 subjects in Arm C have completed the first cycle, • at any other time as needed following random assignment in Part 2. The safety run-in of Arm C (n=3) will occur with a 1-week interval between dosing of subjects and an SRC will convene after all subjects have received 1 cycle of therapy. The SRC has the authority to recommend dose or regimen 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.	Simplify language and clarify the role of the SRC

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Additional sub-investigators and personnel may participate in reviews, as appropriate. Following a treatment arm review, the SRC may recommend proceeding with enrolling additional subjects in the current treatment arm or not enrolling any additional subjects. 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 at any time.	
Section 5.1.4 Enrollment Procedure	**Revised Text:** During **Part 1**, 6 eligible subjects will enroll into Arm A, followed by 3 subjects in Arm C. During **Part 2**, eligible subjects will be randomly assigned by the Sponsor or its representative: Subjects will be adaptively randomized in a 1:1 ratio to receive Ad-RTS-hIL-12 monotherapy (Arm A) or combination therapy (Arm C) according to the current status of the treatment arms and whether or not a subject has consented to the "Additional Immunologic Assessments" until the desired number of subjects is fully enrolled in each study arm.	Clarify enrollment procedure for updated study design
Section 5.1.5 Treatment Arm Expansion Procedure	**Revised text:** Should a subject discontinue study treatment before receiving 6 cycles for reasons other than progression, another subject may be added to the treatment arm to allow for full analysis. Response evaluation and criteria for futility are described in Section 9 Statistical Methods. Subject enrollment and expansion of treatment Arms A and C will proceed according to a standard Simon 2-stage design (Table 1). An alternate dose level of palifosfamide and/or INXN-1001 may be explored after review of all available safety and activity data, and will be communicated by an administrative letter and enacted upon issuance.	Clarify treatment arm expansion procedure for updated study design
Section 7.2.3 Treatment Regimen	**Revised text:** A combination of up to 3 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 (once daily doses on Days 1-7) in	Update treatment regimen Clarify importance of and recommendation for

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	combination with an IT injection of INXN-2001 (on Day 1 of each cycle). For each 21-day treatment cycle, INXN-2001 dosing will occur 3 hours ± 30 minutes after the first dose of INXN-1001. INXN-2001 will be given as IT injections [redacted] in 1 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 an alternate lesion or a draining, pathologic lymph node of a previously accessible lesion. A pathologic lymph node must be defined as a lesion per mRECIST (Appendix 4). 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 must be instructed to maintain good oral hydration on and between dosing days. Oral hydration (approximately 2000 mL oral electrolyte solution per 24 hours) and administration of prophylactic antipyretics is highly recommended for 72 hours following injection of INXN-2001 because most toxicity (including intense fevers, chills, fatigue and dehydration) has been observed during this time period. Blood pressure should be monitored regularly. For treatment Arm C Combination Therapy (Ad-RTS-hIL-12 + palifosfamide), palifosfamide will be administered following administration of INXN-1001 as a 30-minute IV infusion, and on Day 1 of each cycle the infusion should complete before injection with INXN-2001. Palifosfamide will be administered intravenously at a dose of 120 mg/m^2/day as a 30-minute infusion on Days 1, 2, and 3 of a 21-day cycle for up to 6 cycles. The temporal sequence of the treatment regimens for study Arms A and C are shown in Figure 4. For subjects enrolled in Arm C Ad-RTS-hIL-12 + Palifosfamide Combination Therapy, the sequence of regimens A + palifosfamide will be followed concurrently as shown below. Figure 4 was revised to display Arms A and C instead of Arms A and B. Arm B has been deleted from the study.	hydration
Section 7.2.4.1 Preparation and Administration of INXN-1001	**Revised text:** 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 (within approximately 30 minutes after the start of a normal meal).	Clarify definition of the fed state

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Section 7.2.4.2 Monitoring of Subject Adherence	**Added text:** Subjects are to be instructed to take all of the capsules in the same way for each of the remaining treatment period, and will be reminded to take their dose via a phone call when they will not have a clinic visit.	Emphasize the importance of adherence
Section 7.2.4.3 Preparation and Administration of INXN-2001	**Added text:** **NOTE**: • All subjects receiving INXN-2001 must be adequately hydrated with approximately 2000 mL per 24 hours of oral electrolyte solution for 72 hours during Cycles 1 and 2. Subjects must be instructed to maintain good oral hydration on and between dosing days.	Clarify/revise inclusion criteria
Section 7.2.4.4 Preparation and Administration of Palifosfamide	**Added text:** **Note: Total cumulative time from reconstitution in vial through completed dose administration should not exceed 2.5 hours (150 minutes).**	Add clarity and detail regarding reconstitution of palifosfamide
Section 7.2.5 Retreatment Criteria, Dose Delays and Modifications	**Added text:** Clinical laboratory tests drawn pre-treatment for analysis on Day 1 of each cycle may be used for determining if the re-treatment criteria have been met, and must be reviewed by the investigator or designee prior to the next cycle of study drug administration. Two treatment delays for recovery to acceptable levels are allowed at weekly intervals up to a maximum of 14 days. In the event that a treatment-related dose delay is necessary for palifosfamide, then the next cycle of study treatment will be administered at 80% of the dose (Table 3). If an AE is clearly related to 1 study drug and not the other (in combination Treatment Arm C), then dose modification/delay is to occur for the relevant study drug (INXN-1001 and/or palifosfamide). A maximum of two dose reductions, of either INXN-1001 or palifosfamide, will be allowed. **Deleted text:**	Clarify dose delays and modifications which may occur for either INXN-1001 or palifosfamide
Section 7.2.6 Severity Grading and Management of Injection Site	**Added text:** **Table 3. Injection Site Reaction Severity Grading and Management**	Update language for clarification

<table>
<tr><th>Section in Amended Protocol</th><th>Revised or Deleted Text/Section</th><th>Rationale for Change</th></tr>
<tr><td>Reactions</td><td>
<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 symptoms (eg, warmth, erythema, itching)</td><td>No intervention required.</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.
If toxicity meets seriousness criteria, immediately report as per SAE reporting procedures; see Section 8.4.</td></tr>
<tr><td>4</td><td>Life-threatening consequences; urgent intervention indicated</td><td>Permanently discontinue study treatment and notify Medical Monitor.
Defaults to “serious”. Immediately report as per SAE reporting procedures; see Section 8.4.</td></tr>
<tr><td>5</td><td>Death</td><td>Immediately notify Medical Monitor and report as per SAE reporting procedures; see Section 8.4.
Discontinue further subject enrollment.</td></tr>
</table>
</td><td></td></tr>
<tr><td>Section 7.2.7
Prophylactic, Antipyretic and/or Analgesic Administration</td><td>NOTE: Since fever and flu-like symptoms (eg, fever, headache, chills, dehydration, etc.) are commonly experienced following adenoviral vector administration, it is highly recommended that subjects be treated with prophylactic, antipyretic and/or analgesic medications prior to study drug administration for the first 72hr of each cycle.</td><td>Clarify symptoms that may necessitate prophylactic antipyretics and/or analgesics and the timing of these</td></tr>
</table>

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Please refer to Appendix 3 for a recommended regimen for the prophylactic administration of antipyretics and/or analgesics.	
Section 7.3 Concomitant Therapy	**Revised text:** • Antiemetics are permitted for study drug-induced nausea and vomiting. (except those listed Appendix 2)	Remove qualifier for antiemetic use
Section 7.4.2.5 Vital Signs	**Added text:** Vital signs will include blood pressure, pulse rate, temperature, and respiration rate. Subject's blood pressure is to be monitored closely, with hydration as needed to prevent hypotension for 72 hours after administration of INXN-2001. Blood pressure assessment is required on Days 1, 2, 3 for Cycle 1 and Cycle 2. [redacted]	Instruction for recommended hydration following dosing with INXN-2001
Section 7.4.2.6 Adverse Events	**Deleted text:**	Deleted redundant text
Section 7.4.2.8 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 7.4.2.12 Immune Response	**Deleted text:**	Simplify and clarify immunologic analyses

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Analysis	Plasma and/or serum cytokine levels will be assayed using enzyme-linked immunosorbant assay (ELISA) methodology. One or more serum biomarkers (eg, MUC-1) or cytokines will be assessed.	
Section 7.4.2.13 INXN-1001 Pharmacokinetic Assessment	**Revised text:** INXN-1001 PK assessment will occur for subjects enrolled in Part 1 during Cycle 1 of study treatment. Whole blood samples will be collected on Day 1, Day 2, Day 7, and Day 8; as defined in Table 2A, Schedule of Assessments. INXN-1001 levels will also be assessed using the samples drawn for cytokine analysis.	Align PK with new dosing regimen
Section 8.4.4 Efficacy Assessments	**Deleted text:** The investigator will evaluate each subject for response to therapy according to mRECIST v1.1 guidelines (see Appendix 4). • **Added text:** 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 anticancer therapy has been initiated, or up to 1 year, whichever occurs first.	Align efficacy assessments with revised study design
Section 8.2.3 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 8.4 Reporting Serious Adverse Events	**Revised text:** SAEs must be reported to the Sponsor or Sponsor's designee ~~immediately~~ within	Revise safety language for clarity

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	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)	
Section 9.1 Determination of Sample Size	**Added text:** In view of up to 3 novel investigational drugs being tested in this study, the sponsor has incorporated a Safety Run-in (Part 1) to evaluate safety for each study arm before fully enrolling all subjects required in Stage 1 of Part 2 for each study arm (for details please see Section 5.1.1).	Update text
Section 9.4.2 Secondary Efficacy Endpoints	**Revised text:** Duration of response: time from the date of first objective response (PR or CR) by mRECIST v1.1, until the date of PD or 1 year.	Clarify the duration of response timeframe
Section 9.4.3 Other ~~Efficacy~~ Endpoints	**Revised text:** Cytokine and breast cancer biomarker (eg, MUC-1) levels will be explored in serum samples. For study sites participating in additional immunologic assessments, please refer to Appendix 6. INXN-1001 PK analysis will be	Simplify and clarify immunologic analyses

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	performed in Cycle 1 for all subjects enrolled the Safety Run-in (Part 1).	
Section 9.6.4.2 Secondary and Other ~~Efficacy~~ Analyses	**Deleted text:**	Simplify and clarify immunologic analyses
Section 9.7 Immunologic Responses	**Added text:** The trial will assess the cytokine profiles and breast cancer-associated biomarkers (eg, MUC-1) for all subjects. In addition, participating sites will include additional immunologic assessments as described in Appendix 6. The immunological effects of each treatment cycle on the cellular and humoral immune responses in the peripheral circulation and in tumor biopsy specimens. Cytokine profiling will be performed by ELISA. Whole blood will be collected for flow cytometry analysis, immune response assay, and/or ELISPOT assays for measurement of the response to breast cancer-associated antigens.	Simplify and clarify immunologic analyses
Section 10.4 Duration of the Study	**Deleted text:** The duration of this study from the time of initiating subject enrollment until the completion of survival follow-up is anticipated to be approximately 34 months, including 24 months for enrollment and the later of 1 year of further follow-up. **Revised text:** f) Follow-Up Tumor Assessment visit performed 35 ± 7 days after the Post-Treatment Safety Assessment visit.	Clarify duration and update to align with dosing regimen and cycle length
Section 13 References	**Deleted references:** 25. 26. 27. 28.	References deleted from deleted Nonclinical studies which are contained in the Investigator's Brochure

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

Section 14.3
Appendix 3
	Added/revised text:
	
	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 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.~~
		Update dosing of antipyretics

Section 14.6 Appendix 6	**Added Appendix 6:** A new Appendix ONLY for study sires participating in additional immunologic	Add a new Appendix to describe additional immunologic analyses that

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	assessments: Schedule of Study Procedures and Assessments - Additional Immunologic Assessments • Biopsy / FNA • PBMC (ie, cellular immune response) • Immune cell evaluation (ie, flow cytometry)	will occur in consented subjects at participating sites

Appendix 1

Palifosfamide dose justification in combination with Ad-RTS-IL-12

The combination of intratumoral (IT) delivery of Ad-RTS-mIL-12 + INXN-1001 generates a localized immune response which requires the careful coordination of a number of signals. An intricate system of checks and balances has evolved to ensure the appropriate response to the localized cytokine stimuli. The activation of naïve T cells in response to a specific antigen or cytokine requires multiple signals including tumor cells own expression of tumor associated antigens. The localized production of IL-12 which results in systemic stimulation of the host immune system, causing an influx of cytotoxic $CD8^+$ T cells coupled with a reduction in $CD4^+$ regulatory T cells. The end result is targeted tumor cytotoxicity as well as the induction of T cell memory resulting in systemic immune modulation.

It is well recognized that responses to many chemotherapeutic drugs are paradoxical since at low doses they actively suppress tumor cell-mediated immunity while at higher doses reduce tumor volume while inducing myelosuppression. Palifosfamide an alkylating agent used in chemotherapy is paradoxical in this regard. The administration of metronomic doses of alkylating agents such as palifosfamide should avoid the potential of immunosuppression while preferentially reducing regulatory T cells and myeloid dependant suppressor cells resulting in priming the immune system.. The balance between palifosfamide-induced cytotoxicity and induction of effective immunity is critical to the success of combination immunotherapy**.**

To test this hypothesis, the effect of Ad-RTS-mIL12 + INXN-1001 in combination with palifosfamide was evaluated in a subcutaneous 4T1 syngeneic BALB/c mouse model. The results of this study demonstrated that combinations of Ad-RTS-mIL-12 + INXN-1001 + palifosfamide 120 mg/m^2 resulted in greater tumor mIL-12 levels concomitant with a reduction in myeloid dependant suppressor cells when compared to corresponding AD-RTS-mIL-12 + INXN1001 or palifosfamide alone. The combination elicited a supra-additive decrease in tumor growth rate over each treatment alone, without inducing toxicity. The increase in tumor IL-12 levels from the combination resulted in a prolonged time for tumor to quadruple (an index of efficacy) greater than the sum of each agent alone resulting in an increased survival greater than the sum of each agent alone (AACR 2012).

In summary, combinations of Ad-RTS-mIL12 + INXN-1001 + palifosfamide 120 mg/m^2 support the hypothesis that metronomic dosing of chemotherapeutic agents augments specific T cell immune response to enhance immunotherapy, which could potentially be translated into a safe clinical regimen for the treatment of metastatic breast cancer.