Clinical Study Protocol

Title:	A Phase Ib Study to Assess the Safety, Tolerability and Immunologic Activity of Preoperative IRX-2 In Early Stage Breast Cancer Patients Protocol: IRX-2 2016-B
US BB IND #:	132055
Investigational Medicinal Product (IMP)	IRX-2 (Leukocyte Derived Cytokine Mixture, Human)
Date:	Original Protocol: August 23, 2016 Amendment #1: June 30, 2017 Amendment #2: January 19, 2018
Principal Investigator	David Page, MD Earle A. Chiles Research Institute 4805 NE Glisan Street, 2N87 Portland, OR 97213 503-215-5696 FAX 503-215-5695 David.Page2@Providence.org
Sponsor:	Providence Portland Medical Center 4805 NE Glisan Street, 2N35 Portland, OR 97213

Investigator Agreement

I have read and approved this protocol. My signature confirms my agreement that **the clinical study will be conducted in full compliance with the protocol and all applicable laws and regulations including, but not limited to, the International Conference on Harmonisation Guideline for Good Clinical Practice (GCP)**, the Code of Federal Regulations (CFR), the ethical principles that have their origins in the Declaration of Helsinki and all applicable Federal and local regulations. All required study information will be archived as required by regulatory authorities.

Nothing in this document is intended to limit the authority of a physician to provide emergency medical care under applicable regulations.

This document is confidential. It contains proprietary information of IRX Therapeutics, Inc. Any disclosure of such information that is not authorized in writing by the sponsor is strictly prohibited. This document must be used solely for the purpose of reviewing or performing this study.

Investigator:		**Date:**	
	David Page, MD		
Institution:	Providence Portland Medical Center		

Protocol Synopsis IRX-2 2016-B

Name of Investigational drug: IRX-2	
Title of Study: A Phase Ib Study to Assess the Safety, Tolerability and Immunologic Activity of Preoperative IRX-2 in Early Stage Breast Cancer	
Study Center(s): Providence Portland Medical Center	
Study Period: 12 months	**Phase of Development:** Phase Ib

Objectives:

Primary objective:

- To establish the safety and tolerability of the IRX-2 regimen when administered pre-operatively in early stage breast cancer (ESBC) patients

Secondary objectives:

- To evaluate for changes in tumor infiltrating lymphocyte (TIL) score associated with the IRX-2 regimen, as measured by H&E TIL count according to Salgado criteria
- In Cohort B: To estimate the pathologic complete response rate to neoadjuvant anthracycline-based and non-platinum containing chemotherapy in patients who have received the IRX-2 Regimen before chemotherapy

Exploratory objectives:

- To evaluate the effect of IRX-2 on additional investigational immunologic and molecular biomarkers

Study Design:

This will be a Phase Ib study conducted to determine the safety and tolerability of an IRX-2 regimen in early stage breast cancer, to be administered pre-operatively before standard-of-care surgical resection and following standard-of-care diagnostic biopsy.

Eligible subjects will have early stage breast cancer of any receptor subtype, for which standard-of-care surgical resection is planned.

Cohort B will enroll subjects triple negative breast cancer (defined by ER<10%, PR<10%, and HER2-negative by NCCN guidelines), T1c+ tumors for which neoadjuvant anthracycline-based and non-platinum containing chemotherapy is planned. The IRX-2 regimen will be administered and completed preceding chemotherapy. Cohort B subjects must undergo post-IRX-2 Regimen biopsy (2-3 cores), followed by commencement of chemotherapy preferably within one week after biopsy.

For all subjects, a minimum of 1 core of pre-IRX2 tumor-bearing biopsy material, either from diagnostic core biopsy or a planned pre-IRX2 research biopsy, must be available for research analysis.

The IRX-2 regimen will be administered in all enrolled subjects according to the schema and dosing schedule delineated in Table 1. IRX-2 will be administered by subcutaneous injection into the periareolar skin of the affected breast by a nurse at Providence Medical Center.

Subjects will be evaluated by physical exam and blood tests at baseline and periodically during the study, culminating in a final study visit 30 days (±7 days) following surgical resection. Safety will be evaluated routinely using the NCI-CTCAE v4.03 toxicity reporting criteria. The toxicity of the IRX-2 has been previously evaluated in two head and neck cancer studies, and therefore no formalized interval safety assessments are planned. However, safety and tolerability of the IRX-2 regimen will be continuously evaluated by the principal investigator in collaboration with the IRX-2 Therapeutics, and the trial may be terminated or held at any time for unanticipated concerns of safety/tolerability.

Therapeutic Regimen

Days of administration*	**Drug**	**Dose**	**Route**	**Comment**
1	Cyclophosphamide	300mg/m^2	IV infusion	Low dose does not require corticosteroids; has low to moderate emetic risk per NCCN Guidelines
Any 10 days between days 4-19	IRX-2	2 mL (230 units of IL-2) on each of 10 days	Two 1 mL subcutaneous periareolar injections	Injections will be administered at Providence Medical Center
1-21	Indomethacin	25mg, TID	Oral	Divided three times daily x 21 days
1-21	Multivitamin with 15-30 mg of zinc/tablet	1 tablet	Oral	Once daily x 21 days
1-21	Omeprazole	1 tablet	Oral	Once daily x 21 days
Ideally 2-5 days after last IRX-2				Clinically indicated breast surgery/biopsy

*As necessitated by scheduling logistics, the oral medications may be discontinued after Day 17 and surgery/biopsy performed 2-12 days later. Indomethacin must be discontinued at least 2 days prior to scheduled surgery/biopsy.

Subjects will be considered evaluable if they consent and receive any part of the cyclophosphamide infusion, but any who are enrolled but withdraw consent prior to beginning the IRX-2 Regimen will not be evaluable and will be replaced. The rate of Grade 1-4 toxicities will be reported as additional, descriptive measures of tolerability. The following criteria must be met for that subject to be considered to have “tolerated” the therapy:

- Subject receives cyclophosphamide, >7 injections of IRX-2 and >10 days of indomethacin, omeprazole and multivitamins with zinc and does not have Grade 3 or 4 treatment-associated adverse events requiring discontinuation of therapy;
- Subject does not experience a delay in standard-of-care surgical resection related to treatment-associated adverse events;
- Subject does not have any ongoing treatment-related Grade 2-4 adverse events at the 30-day post-operative follow-up visit;
- Subject does not withdraw consent after the start of the cyclophosphamide infusion.

Because this therapy is being administered in subjects receiving therapy intended to cure their disease, the threshold for establishing safety/tolerability will be high, such that nearly all subjects must tolerate the therapy. The therapy will be deemed “safe and tolerable” if 75% or greater of evaluable subjects tolerate the therapy according pre-specified definition of tolerability (see Section 7.2 for additional detail).

The secondary endpoint is to evaluate the effect of therapy on stromal TIL (sTIL) infiltration by Salgado criteria. The rationale for this endpoint is that the percentage of TILs in pre-operative biopsies is highly correlated with both overall survival and response to neoadjuvant therapy. If the IRX-2 regimen is found to increase the percentage of TILs, this would provide rationale to evaluate further IRX-2 in this setting.

Exploratory endpoints will be analyzed descriptively, and will include additional peripheral blood and intratumoral analyses of the immunologic effects of the IRX-2 regimen, including, for example, some or all of the following:

- flow cytometric quantification and characterization of peripheral lymphocytes (including activated T-cells, T-regulatory cells, NK cells, and myeloid cells)
- TIL phenotype characterization by multispectral immunofluorescence imaging to assess changes in abundance of T-regulatory cells, activated T-cells, myeloid lineages and dendritic cells
- Intratumoral and T-cell clonal responses by T-cell receptor DNA deep sequencing
- Characterization of intratumoral immune responses by RNA expression using the Nanostring PanCancer Immune panel and/or Prosigna tumor recurrence score

Based upon conservative estimates of enrollment of 2 subjects per month, the accrual time is estimated to be 1 year.

Criteria for Inclusion in the Study:

Inclusion Criteria

1. Invasive breast cancer of any receptor subtype diagnosed by core-needle biopsy
2. To undergo surgical resection with curative intent by partial mastectomy (lumpectomy) or mastectomy
3. Tumor >5 mm in maximum diameter by ultrasound or mammography. (Subjects with smaller tumors may be included at the discretion of the Principal Investigator.) Cohort B: (defined by ER<10%, PR<10%, and HER2-negative by NCCN guidelines), T1c+ tumors for which neoadjuvant anthracycline-based and non-platinum containing chemotherapy is planned.
4. Willing and able to provide written informed consent, including consent for use of

available tissue and required blood draws for research purposes
5. Availability of at least one tumor-bearing core specimen from the breast cancer diagnostic biopsy
6. Performance status of KPS 70% or greater.
7. Female or male ≥18 years of age on day of signing informed consent.
8. Adequate organ function, defined as
    - Absolute neutrophil count ≥1,500/μL.
    - Platelet count ≥100,000/μL.
    - Serum creatinine ≤1.5 × upper limit of normal (ULN) or CrCl ≥60 mL/min.
    - AST and ALT ≤2.5 × ULN
    - Bilirubin ≤1.5 × ULN or direct bilirubin ≤ULN. (Not applicable in patients with Gilbert's Syndrome.)
    - INR/PT and PTT ≤1.5 × ULN
    - Negative serum or urine pregnancy test if of childbearing potential
9. Women of childbearing potential must use acceptable measures to avoid becoming pregnant while on treatment and for 1 year after last dose of investigational therapy.

Exclusion Criteria

1. Neoadjuvant systemic therapy is planned (except for Cohort B subjects)
2. Prior surgery, radiotherapy or chemotherapy for this cancer (other than core-needle biopsy)
3. Received an investigational agent within 4 weeks of the first dose of treatment.
4. Diagnosis of immunodeficiency or has received more than replacement doses of corticosteroids any other immunosuppressive therapy within 4 weeks of the first dose of treatment
5. Hypersensitivity to IRX-2, cyclophosphamide, indomethacin, aspirin or ciprofloxacin.
6. Chronic anticoagulation, not including aspirin, but including heparins, warfarin, oral anticoagulants or other platelet function inhibitors, that cannot, in the documented opinion of the investigator, safely be interrupted from at least 2 days prior to the initiation of the study regimen until after surgical resection of the tumor.
7. Another malignancy that required active treatment within 6 months of the first dose of treatment
8. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, such that trial participation is not in the best interest of the subject, including but not limited to uncontrolled hypertension or clinically significant cardiovascular disease, myocardial infarction within the previous 3 months, active infection or pneumonitis or other pulmonary disease requiring systemic therapy, clinically significant gastritis or peptic ulcer disease (that would preclude the use of indomethacin), stroke of other symptoms of cerebral vascular insufficient within the last 3 months, autoimmune disease that has required systemic treatment within the past 2 years (other than hormone replacement doses), or uncontrolled psychiatric or substance abuse disorders.
9. Pregnancy or lactation.
10. Known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

Number of Subjects: 39 anticipated

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

List of Definitions and Abbreviations

AE	adverse event
AJCC	American Joint Committee on Cancer
Akt	protein kinase B
ALT/SGPT	alanine aminotransferase/serum glutamic pyruvic transaminase
ANC	absolute neutrophil count
AST/SGOT	aspartate aminotransferase/ serum glutamic oxaloacetic transaminase
BUN	blood urea nitrogen
CBC	complete blood count
CD	cluster of differentiation
CFR	Code of Federal Regulations
CRF	case report form
CRO	Contract Research Organization
CT	computed tomography
DFS	disease-free survival
DSMB	Data and Safety Monitoring Board
EFS	event-free survival
ESBC	early stage breast cancer
EU	European Union
FDA	Food and Drug Administration
FDG-PET	fluorodeoxyglucose positron emission tomography
Fox P3	Fox P3 protein is involved in immune system responses
GM-CSF	granulocyte-macrophage colony stimulating factor
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
HR	hazard ratio
IB	Investigator's Brochure
IEC	Independent Ethics Committee
IFN-α	interferon alpha
IFN-γ	interferon gamma
IL-1β	interleukin 1 beta
IL-2	interleukin 2
IL-7	interleukin 7
IL-10	interleukin 10
IL-12	interleukin 12
IL-15	interleukin 15

IRB	Institutional Review Board
IRX-2	primary cell-derived biologic with multiple active cytokine components
IRX-2 regimen	Twenty subcutaneous injections of 115 U of IRX-2 (over 10 days) preceded by cyclophosphamide with a 21 day course of indomethacin and zinc containing multivitamins
ITT	Intention-To-Treat
IV	intravenous
IxRS	Interactive Recognition System
KPS	Karnofsky performance status
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MHC	major histocompatibility complex
MHC class I	major histocompatibility complex class 1
MHC class II	major histocompatibility complex class 2
MRI	magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK or NKT	natural killer T cells
OS	overall survival
PET	positron emission tomography
PDL1	programmed death ligand 1
PHA	Phytohemagglutinin
PI	Principal Investigator
PI3K	phosphoinositide 3 kinase
PT	prothrombin time
PTT	partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SAP	statistical analysis plan
SCC	squamous cell carcinoma
T cells	cellular immune lymphocytes; derivation – thymus lymphocytes
T-bet	T box transcription factor
TCR	T cell receptor
T_{eff}	T effector cell
TGF-β	Transforming growth factor beta
T_{reg}	T regulatory cell; suppressor T cell
TID	three times a day

TIL	tumor infiltrating lymphocytes
TNF-α	tumor necrosis factor alpha
TNM	tumor grading system (T-primary lesion; N-nodes; M-metastases)
U	unit(s)
ULN	upper limit of normal
US	United States

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Breast Cancer

The American Cancer Society estimates that approximately 40,000 people in the USA will die annually of metastatic breast cancer [American Cancer Society, 2012]. Breast cancer ranks second as the cause of cancer deaths in women, and while deaths rates are falling, patients diagnosed with advanced breast cancer remain at high risk of dying from their disease.

Immunotherapy to facilitate immune recognition of micrometastatic disease has been of scientific interest, particularly in patients at high risk of tumor recurrence. There are numerous vaccination and immunotherapy trials listed on clinical trials.gov that are open for the adjuvant or neo-adjuvant treatment of patients with breast cancer. Examples of vaccines being evaluated include a peptide Her2/neu vaccine, vaccines against carcinoembryonic antigen (CEA) and cancer testis antigens (CTA). These vaccines also include various adjuvant components designed to optimize immune response, for example, a toll-like receptor 3 agonist, GM-CSF, or dendritic cells. In addition to vaccines, other immunotherapeutic approaches, for example immune checkpoint blockade with ipilimumab or tissue-destructive in situ vaccination approaches such as tumor cryoablation [Page, 2014] are also being evaluated.

Specifically for triple negative breast cancer, the standard-of-care is to consider pre-operative neoadjuvant chemotherapy. Benefits of this approach are to assess for complete response, which is prognostic for overall survival, and to potentially surgically downstage. Because immune infiltrates are predictive to response to chemotherapy in this population, introduction of immunotherapy either before or during chemotherapy is of potential therapeutic utility.

1.1.2 The Role of the Immune System in Breast Cancer

The tumor microenvironment and tumor immunogenicity have recently been recognized as a contributing component in pathogenesis and response to therapy. Recently published studies suggest that distant metastatic invasion of breast cancer, specifically “triple negative breast cancer” (TNBC) may be induced by immune and/or inflammatory deregulation. Among a set of 45-gene signatures that was found in distant disease recurrence, ten genes found within the transforming growth factor-β signaling pathway are associated with an immune-suppressed phenotype [Kuo, 2012]. In patients with breast cancer, either a decreased number of CD8+ T-cells, or an increased number of regulatory T-cells, was associated with adverse tumor characteristics such as lymph node metastasis, higher stage, and Ki-67 immunopositivity [Miyashita, 2014]. Corroborating these findings, across multiple large adjuvant and neoadjuvant datasets, the quantity of tumor-infiltrating lymphocytes (TILs) in early-stage TNBC has been associated with improved survival, and increased likelihood of response to chemotherapy [Miyashita, 2014; Denkert, 2010]. A study from the German Breast Group addressed the role of TILs in predicting response to neoadjuvant therapy with anthracycline and taxane chemotherapy [Denkert, 2010]. The percentages of intratumoral and stromal lymphocytes in pretreatment tumor biopsies were identified as significant independent predictors of pCR in both a training (n=218) and validation cohort (n=840) (p=0.012; -0.001). Additionally, pretreatment TILs have been linked to improved DFS and OS in breast cancer [Adams, 2014]. Functional assays demonstrate that adequate activation of TILs derived from breast cancer tissue could restore the appropriate antitumor immune responses [DeNardo, 2007]. This emerging data underscores the potential of a therapeutic strategy that increases immune-mediated antitumor activity. Therefore, a reasonable hypothesis to examine is that modulation of the immune system might improve outcomes.

1.1.3 Prognostic significance of tumor infiltrating lymphocytes

The quantity of TILs within breast cancer tumor stroma has been validated across multiple adjuvant studies as a strong and independent predictor of recurrence [Adams, 2014]. A consensus methodology of scoring stromal TILs has been developed by an international breast cancer TILs working group, and is being prospectively validated [Salgado, 2014]. The methodology involves scoring of lymphocytes on a single tumor-bearing H&E slide on a scale of 0-100% lymphocytes. Using this methodology in a pooled analysis of 5 adjuvant triple negative chemotherapy trials (n=991), stromal TILs were prognostic for invasive disease free survival (HR=0.84, 95% CI 0.78-0.91) and overall survival [Loi, 2015]. Furthermore, stromal TILs were found to be independently prognostic when analyzed in conjunction with clinical and pathologic variables including age, tumor size, and node positivity by multivariate cox analysis. In subjects with matched lymph node status, increases in TILs by 20% were associated with improvements in 5-year distant relapse free survival rates as great as 10%.

In light of these data, there is strong rationale to evaluate immunotherapy techniques that may increase stromal lymphocyte infiltration, and potentially improve outcomes, in patients with early stage breast cancer in pre-operative clinical trials.

1.1.4 Immune Deficiency and Immunotherapy in Cancer Patients

The rationale for the pursuit of immunotherapy in patients with cancer stems from a considerable body of evidence, which indicates that immunologically competent cells are important host defense mechanisms. Many human cancers, however, are associated with cellular immunodeficiency. Immunotherapies now in development for the treatment of multiple cancers include nonspecific, systemic cell-mediated approaches, cytokine based immunotherapy (including delivery of granulocyte-macrophage colony stimulating factor [GM-CSF], interleukin-2 [IL-2], interferon alpha [IFN-α], interferon gamma [IFN-γ], interleukin-12 [IL-12], and IRX-2), tumor-associated antigens as therapeutic targets, monoclonal antibodies and cancer vaccines, either peptide/protein based, dendritic-cell based or nucleic acid based, and immune checkpoint inhibitors. Discussion of these many approaches is beyond the scope of this protocol Introduction but is presented in more detail in the Investigator's Brochure (IB) and in several reviews [

Petrelli F, Coinu A, Borgonovo K, Cabiddu M, Ghilardi M, Lonati V, Barni S. The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat 2014 144:223-32.

Rapidis, 2009; Freiser, 2013; Gildener-Leapman, 2013].

1.1.5 The IRX-2 Regimen

The concept that subjects with cancer might benefit from immune stimulation led to the development of the IRX-2 regimen, first studied in patients with head and neck squamous cell cancer (HNSCC), a malignancy clearly associated with immune deficiency [Hadden, 1994]. The IRX-2 regimen consists of a cell-derived biologic, IRX-2, that contains multiple cytokines, cyclophosphamide, indomethacin, omeprazole, and multivitamins with zinc. These four components have been administered in patients with HNSCC as a regimen consisting of an intravenous (IV) infusion of low-dose cyclophosphamide on Day 1, followed by oral indomethacin, omeprazole and oral multivitamins with zinc daily for 21 days, and IRX-2 administered as two subcutaneous injections per day for ten days [Freeman, 2010; Wolf, 2011]. Omeprazole, a proton pump inhibitor, is given concurrently to improve tolerance of the indomethacin.

1.1.6 Manufacture and Composition of IRX-2

IRX-2 is a primary cell-derived biologic with multiple active cytokine components produced under pharmaceutical standards as discussed in more detail in the IB. Briefly, human leukocytes ("buffy coats") pooled from multiple donors are stimulated with phytohemagglutinin and ciprofloxacin. Subsequently, the phytohemagglutinin, ciprofloxacin and all cellular elements are removed or significantly reduced, and the cell-free supernatant is filter sterilized, nanofiltered to clear viral particles, vialed, and frozen as IRX-2.

IL-2 is the major cytokine in IRX-2, followed by IFN-γ, TNF-α, and interleukin 1 beta (IL-1β). These cytokines when studied individually enhance cell-mediated immunity via several different mechanisms discussed below and in the IB.

1.1.7 Mechanism of Action of IRX-2

Recent in vitro studies have elucidated several potential mechanisms of action of IRX-2, and these various mechanisms of action need not be exclusive. IRX-2 treatment of human monocyte-derived dendritic cells results in changes consistent with the development of mature activated dendritic cells. Specifically, IRX-2 increased the percentage of cells expressing CD83 and CCR7, markers for dendritic cell maturation and migration and increased the expression of multiple markers that are critical mediators of T cell activation [Egan, 2007]. Similar results were obtained in a later study in cells obtained from patients with HNSCC [Schilling, 2013]. Also, in an in vitro study of peripheral blood mononuclear cells obtained from patients with HNSSC, IRX-2 up-regulated cytotoxicity of NK cells and did so more effectively than IL-2 [Schilling, 2012].

IRX-2 can also protect T cells from activation induced cell death by reversing microvesicle induced inhibition of the PI3K/Akt pathway and correcting the imbalance of pro- versus anti-apoptotic proteins induced by tumor-derived microvesicles [Czystowska, 2009; Czystowska 2011]. IRX-2 was superior to recombinant IL-7 and IL-15 in protecting T cells from tumor-induced apoptosis. The presence of IRX-2 in a tumor microenvironment model promoted the induction and expansion of IFN-γ^{+}T-bet^{+} T_{eff} and significantly decreased the induction of inducible IL-10^{+}TGF-β^{+} T_{reg}. The responsible mechanism involved IFN-γ^{+}-driven T cell polarization towards T_{eff} and suppression of T_{reg} differentiation [Schilling, 2012]. In the Phase 2a study in subjects with HNSSC (described below in Section 1.3.1), IRX-2 mediated reductions in circulating B and NKT cell numbers, suggesting redistribution of these cells to tissues [Whiteside, 2012]. A decrease in naïve T cells was also noted, suggesting their upregulation to memory T cells, while unchanged numbers of T_{regs} (suppressor T cells) after IRX-2 therapy indicated that IRX-2 does not expand this compartment, potentially benefiting anti-tumor immune responses [Whiteside, 2012].

Finally, IRX-2 has been shown to induce enhanced T cell responses when administered with tumor antigen vaccines, raising the possibility that IRX-2 treatment in subjects with HNSCC enhances endogenous antigen-specific T cell responses to the tumor [Naylor, 2010].

1.1.8 Rationale for the Components of the IRX-2 Regimen

The rationale for the components of the IRX-2 regimen is outlined below.

Cyclophosphamide: One mechanism for reversal of anergy and reversal of suppression of immune responses in subjects with malignancy by adoptive immunotherapy may be related to inhibition of regulatory T cell function [North, 1982; North, 1984]. Evidence indicates that cyclophosphamide inhibits T_{reg} number and/or function [Emens, 2005]. Thus many clinical trials that involve immunotherapy or attempt to stimulate immune response to tumor antigens have employed low dose cyclophosphamide (300 mg/m^2) as a component of the treatment regimen. This immunomodulatory dose is less than one-third of a typical anti-cancer dose and is intended

to enhance the development of cell-mediated immunity by providing contrasuppression of tumor-associated immune suppression (to reduce the number and function of regulatory T cells, i.e. T_{reg}) [Berd, 1982; Machiels, 2001].

Pathways of local immune tolerance, escape mechanisms active within the tumor microenvironment and superimposed potent systemic mechanisms of immune tolerance have been reviewed [Emens, 2005] and are discussed in more detail in the Investigator's Brochure (Section 3.4.1). The use of cytotoxic chemotherapy in doses and schedules designed to abrogate specific mechanisms of immune tolerance in order to release the full potential of an antitumor immune response is discussed. Specifically, cyclophosphamide may be used to prime the immune system by promoting the differentiation of CD4+ T helper cells and by abrogating the suppressive influence of CD4+CD25+ T regulatory (T_{reg}) cells. In the absence of T_{reg} influence, high-avidity CD8+ T cells are recruited to an antigen-specific immune response. Cyclophosphamide also facilitates the establishment of memory CD8+ T cells. Thus inclusion of cyclophosphamide in combination with other immune-modulatory agents is supported by both pre-clinical and clinical data as reviewed in more detail by Emens (2005) and in the Investigator's Brochure (Section 3.4.1).

Indomethacin: Indomethacin, a nonselective COX-1/COX-2 inhibitor, is a potent inhibitor of prostaglandin synthesis and may reverse the immunosuppression induced by prostaglandin [Lapointe, 1992; Hadden, 1994]. Numerous experimental, epidemiologic, and clinical studies suggest that non-steroidal anti-inflammatory drugs, including indomethacin, suppress cyclooxygenase and have promise as anticancer agents, particularly for chemoprevention of and as adjuvant therapy in patients with cancer [Thun, 2002; Investigator's Brochure, Section 3.4.2].

Zinc with multivitamins: Subclinical zinc deficiency is common in subjects with HNSCC, perhaps related to a history of alcohol consumption [Brookes, 1981]. The importance of zinc in cellular immunity has been described and several reviews are available [Good, 1979; Keen, 1990; Blewett, 2012; Haase, 2014]. Alcohol consumption is frequently associated with nutritional deficiency, which can also result in impaired immune response [Słotwińska, 2014; Bianchini, 2012; Wintergerst, 2007]. Thus, based on these observations and the lack of any contraindication to their use, zinc-containing multivitamins have been added to the IRX-2 regimen.

Omeprazole: Omeprazole, a proton pump inhibitor, is active at preventing indomethacin-induced gastritis. It is administered with the IRX-2 regimen to decrease the likelihood of indomethacin-induced gastritis.

1.1.9 Delivery of IRX-2

The route of administration of IRX-2 takes advantage of the normal pathways of lymph node activation. Normally, lymphatics drain from an area of disease, such as a tumor bed, and antigens and other factors associated with disease migrate in the lymphatics to the regional nodes. By presenting the cytokine-containing biologic in the area of the tumor-draining lymph nodes rather than systemically, there is an opportunity to mobilize antigen presenting cells and enhance dendritic cell function as well as directly activate T cells to proliferate and become cytotoxic lymphocytes. Additionally, subcutaneous administration has been less toxic since the systemic cytokine drug concentration is much lower.

In this study, we will evaluate the feasibility of injection of the cytokine mixture, IRX-2, subcutaneously into the periareolar skin of the affected breast. Periareolar injection of radioactive colloid is an acceptable and clinically utilized technique for localizing sentinel lymph nodes in breast cancer [McMasters, 2011] and therefore it is assumed that periareolar injection of IRX-2 will be a feasible, acceptable and effective method of delivering the study agent to the draining lymph nodes of the tumor.

1.2 Nonclinical Studies with the IRX-2 Regimen

Nonclinical studies have demonstrated an effect of IRX-2 on T cells in animal models as well as in cellular assays. These studies are summarized briefly in Section 1.1.7 above and in more detail in the IB.

1.3 Clinical Experience with the IRX-2 Regimen in Subjects with HNSCC

1.3.1 Phase 1 Trial

IRX-2 2004-B was a multicenter, Phase 1 trial in subjects with advanced stage HSNCC who had progressed after surgery and/or radiation therapy that was designed to evaluate the clinical and laboratory safety and tolerability of the IRX-2 regimen. Results of the study are presented in the IB and have been published [Freeman, 2010]. The reported toxicities were acceptable overall and did not preclude proceeding to additional trials.

1.3.2 Phase 2 Trial

1.3.2.1 Study Design and Subject Demographics

IRX-2 2005-A was a multi-center trial entitled “A Phase 2, Open-label Trial of the Safety and Biological Effect of Pre-operative Subcutaneous IRX-2 (with Cyclophosphamide, Indomethacin, and Zinc) in Subjects with Resectable Cancer of the Head and Neck.” Results of the study are presented in the IB and have been published [Wolf, 2011; Berinstein, 2012; Whiteside, 2012] and are also summarized here.

The study objectives were to determine the safety of the IRX-2 regimen when used as neoadjuvant (preoperative) therapy and to evaluate clinical, pathological, and radiographic tumor response and disease-free survival (DFS) and overall survival (OS).

1.3.2.2 Clinical Results

1.3.2.2.1 Radiographic Changes:

Changes in tumor size between baseline and immediately pre-operative, i.e. after the IRX-2 regimen, measurements were evaluable in 23 subjects. Based on measurement of the longest single tumor diameter, tumor growth or shrinkage was as follows: 4 subjects had a decrease in tumor size, 16 subjects had no significant change, and 3 subjects had an increase in tumor size [Wolf, 2011]. Review of the radiologic findings in relation to the pathology finding in the resection specimens, however, established that lymph node or tumor enlargement due to reactive hyperplasia could not be distinguished from enlargement related to tumor growth [Wolf, 2011].

One subject underwent a fluorodeoxyglucose positron emission tomography (FDG-PET) CT scan at baseline and at completion of the IRX-2 regimen. Elevated glycolytic activity was observed in 2 lymph nodes and in the primary tumor on the baseline PET scan. At the completion of the IRX-2 regimen, there was a 75% decrease in glycolytic activity in these lesions [Wolf, 2011].

1.3.2.2.2 Disease-free and Overall Survival:

After 5 years of follow-up, median DFS and OS had not been reached. These results for both DFS and OS appeared to be slightly superior to those observed in a comparable group of 81 historical controls, treated at the University of Michigan and matched for baseline characteristics [G. Wolf, personal data].

1.3.2.3 Safety Results

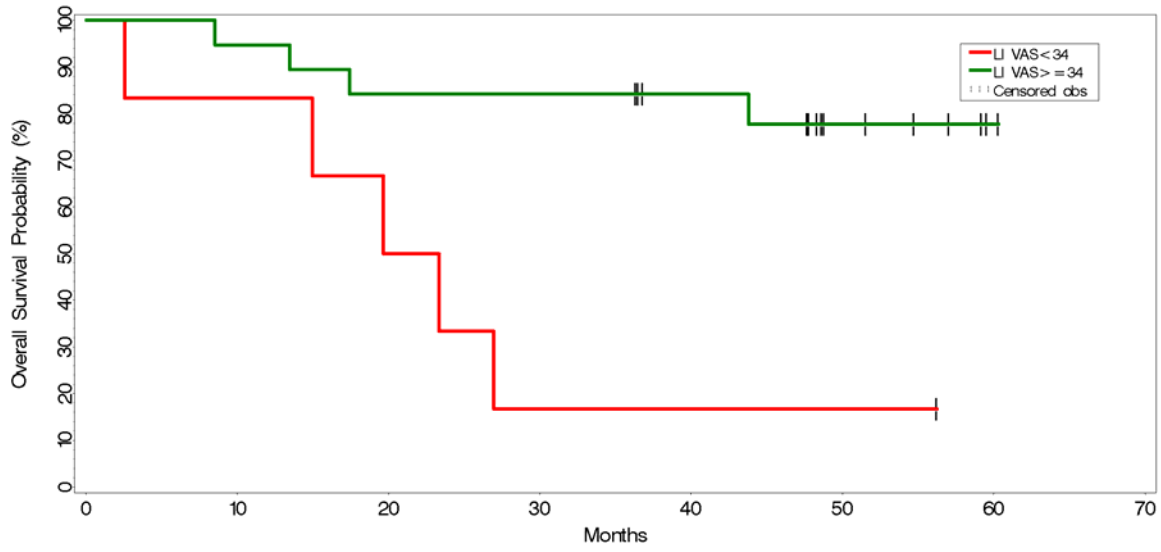
The IRX-2 regimen was tolerated with minimal toxicity. Compliance was excellent; all subjects completed the regimen and there were no unplanned delays in surgery as a result of the immunotherapy regimen. There were no reports from investigators of any unanticipated or unusual delays or difficulties in performing the planned resections or reconstructions or during the postoperative recovery.

The most common adverse events (AEs) were headache (30%), injection site pain (22%), nausea 22%), constipation (15%), dizziness (15%), fatigue (11%), aspiration pneumonia (11%), anemia (11%) and myalgia (7%). All were Grade 1-2 except for the aspiration pneumonias (one Grade 3, one Grade 4) and all resolved without sequelae. There were only minor (Grade 1) alterations in post-treatment laboratory values. Eight serious adverse events (SAEs) in 7 subjects were reported during treatment and the 30-day post-operative period: aspiration pneumonia (n = 3), respiratory tract infection, asthma exacerbation, wound infection, neck abscess and alcohol withdrawal (n = 1 each); only the postoperative wound infection was considered related to the study treatment [Wolf, 2011]. Note that many of these AEs, including e.g. aspiration pneumonia, wound infections, alcohol withdrawal, are common in HNSCC subjects during their pre- and post-operative course. During treatment, several subjects noted decreased pain or improved swallowing and no significant progressive symptoms were noted.

1.3.2.4 Immunologic Results

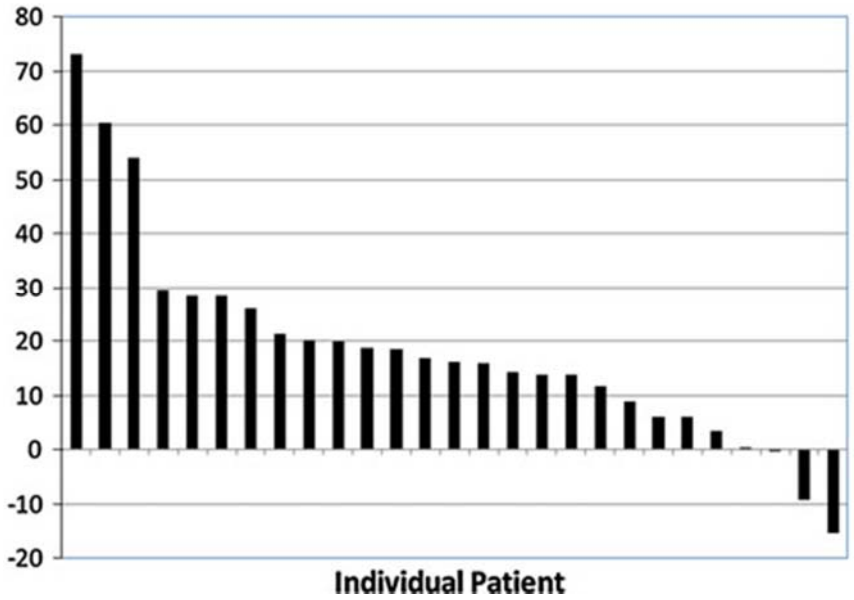
Pretreatment tumor biopsies and the tumor surgical specimens from 25 subjects were characterized for lymphocyte infiltration, necrosis and fibrosis using both hematoxylin and eosin stains and immunohistochemistry [Berinstein, 2012]. Kaplan-Meier estimates of overall survival are displayed in Figure 1 as a function of high and low lymphocyte infiltration in the surgical specimens after the IRX-2 immunotherapy. Eighteen subjects were in the better survival group and 7 were in the inferior survival group; the survival curves are significantly different ($p < 0.05$) [Berinstein, 2012].

Figure 1 Overall Survival by High and Low Lymphocyte Infiltration in the Surgical Resection Specimen (Study IRX-2 2005-A)



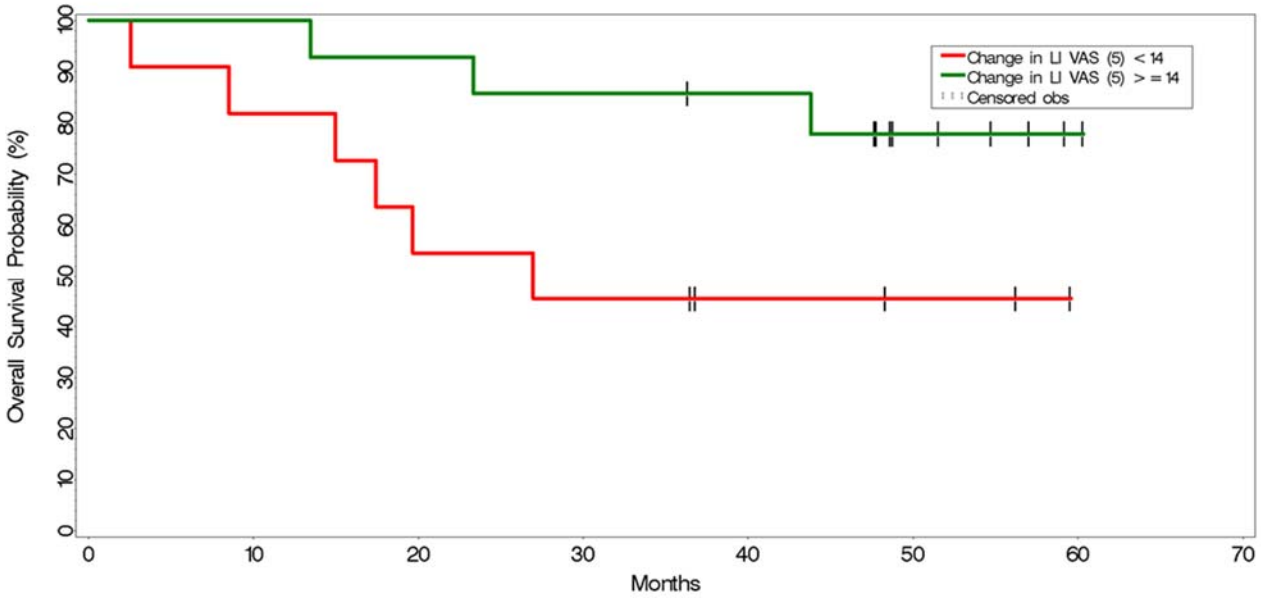
When lymphocyte infiltration in the pretreatment biopsies was compared to that in the resected surgical specimen, increases in lymphocyte infiltration were seen as shown in Figure 2 (change in mean lymphocyte infiltration from the biopsy to the surgical specimen is shown on the y-axis) [Berinstein, 2012].

Figure 2 Lymphocyte Infiltration (Study IRX-2 2005-A)



In addition, subjects in whom the greatest increase in tumor lymphocyte infiltration from biopsy to surgery (n=14) was observed had a trend toward superior survival compared to subjects in whom no or more limited change was observed (n=11) as shown in Figure 3 (p = 0.10) (IRX Therapeutics, unpublished observations).

Figure 3 Overall Survival by Change in Lymphocyte Infiltration from Biopsy to Resection Specimen (Study IRX-2 2005-A)



Peripheral blood lymphocyte subsets also were monitored pre- and post-treatment with the IRX-2 regimen to evaluate changes induced by the IRX-2 regimen (summarized in Section 1.1.7 above and in the IB). The IRX-2 regimen-mediated reductions in B and NKT cell numbers in the blood suggested a redistribution of these cells to tissues while the unchanged numbers of T_{regs} after IRX-2 therapy indicated that IRX-2 does not expand this compartment, potentially benefiting anti-tumor immune responses [Whiteside, 2012].

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective is to establish the safety and tolerability of the IRX-2 regimen when administered pre-operatively in early stage breast cancer (ESBC) patients.

2.2 Secondary Objective

- To evaluate for changes in tumor infiltrating lymphocyte (TIL) score associated with the IRX-2 regimen, as measured by H&E TIL count according to Salgado criteria [Salgado, 2014].
- In Cohort B: To estimate the pathologic complete response rate to neoadjuvant anthracycline-based and non-platinum containing chemotherapy in patients who have received the IRX-2 Regimen before chemotherapy

2.3 Exploratory Objective

The exploratory objective is to evaluate the effect of IRX-2 on additional investigational immunologic and molecular biomarkers.

Exploratory endpoints will be analyzed descriptively, and will include additional peripheral blood and intratumoral analyses of the immunologic effects of the IRX-2 regimen, including, for example, some or all of the following:

- flow cytometric quantification and characterization of peripheral lymphocytes (including activated T-cells, T-regulatory cells, NK cells, and myeloid cells)
- TIL phenotype characterization by multispectral immunofluorescence imaging to assess changes in abundance of T-regulatory cells, activated T-cells, myeloid lineages and dendritic cells
- Intratumoral and T-cell clonal responses by T-cell receptor DNA deep sequencing
- Characterization of intratumoral immune responses by RNA expression using the Nanostring PanCancer Immune panel and/or Prosigna tumor recurrence score

3 INVESTIGATIONAL PLAN

3.1 Study Design

This will be a Phase Ib study conducted to determine the safety and tolerability of an IRX-2 regimen in early stage breast cancer to be administered pre-operatively before standard-of-care surgical resection and following standard-of-care diagnostic biopsy, and patients with triple negative breast cancer who will receive the IRX-2 regimen preceding standard of care neoadjuvant chemotherapy.

Eligible subjects will have early stage breast cancer of any receptor subtype, for which standard-of care surgical resection is planned, or T1c+ triple negative breast cancer with planned standard of care neoadjuvant chemotherapy treatment (anthracycline-containing and non-platinum containing).

To be eligible, a minimum of 1 core of tumor-bearing biopsy material must be available for research analysis.

The IRX-2 regimen will be administered in all enrolled subjects according to the schema and dosing schedule delineated in Table 1. IRX-2 will be administered by subcutaneous injection by study personnel at Providence Medical Center.

Subjects will be evaluated by physical exam and blood tests at baseline and periodically during the study, culminating in a final study visit 30 days (+/- 7 days) following surgical resection. Safety will be evaluated routinely using the NCI-CTCAE v4.03 toxicity reporting criteria.

All subjects are to receive standard of care surgery and postoperative adjuvant radiation or chemo- or hormonal therapy, as appropriate, or for triple negative breast cancer patients, neoadjuvant treatment with chemotherapy.

Table 1 Therapeutic Regimen

Days of administration*	Drug	Dose	Route	Comment
1	Cyclophosphamide	300mg/m2	IV infusion	Low dose does not require corticosteroids; has low to moderate emetic risk per NCCN Guidelines
Any 10 days between days 4-19	IRX-2	2 mL (230 units of IL-2) on each of 10 days	Two 1 mL subcutaneous periareolar injections	Injections will be administered at Providence Medical Center.
1-21	Indomethacin	25mg, TID	Oral	Divided three times daily x 21 days
1-21	Multivitamin with 15-30 mg of zinc/tablet	1 tablet	Oral	Once daily x 21 days
1-21	Omeprazole	1 tablet	Oral	Once daily x 21 days
Ideally 2-5 days after last IRX-2				Clinically indicated breast surgery, or post-IRX2 biopsy

*As necessitated by scheduling logistics, the oral medications may be discontinued after Day 17 and surgery or biopsy performed 2-12 days later. Indomethacin must be discontinued at least 2 days prior to scheduled surgery or biopsy.

Adverse events will be collected from Day 1 (day of cyclophosphamide) until the day of surgery. A final study visit 30 days (±7 days) following surgical resection any AEs felt by the investigators to be definitely or possibly related to the study regimen.

Serious adverse events (not including hospitalization required for breast surgery) will be collected from Day 1 until 30 days after surgery. All SAEs will be tabulated regardless of relatedness to study drugs.

3.1.1 Rationale for the Primary Objective

No significant safety concerns have been recognized in prior Phase 1 and Phase 2a clinical trials of the IRX-2 regimen in subjects with HNSCC. Nevertheless, both trials included only subjects with HNSCC, were relatively small trials, and involved injection into a different anatomical site (i.e. the cervical neck nodal basin). Therefore, this trial is needed to establish the safety of the IRX-2 regimen in subjects with early stage breast cancer using an approach of periareolar sub-cutaneous injection. The safety of IRX-2 will be determined by evaluation of AEs and assessment of any surgical delays associated with administration of the study regimen.

The rationale of cohort B is to estimate safety and immunologic effects in the pre-neoadjuvant chemotherapy setting.

3.1.2 Rationale for the Secondary Objectives

Across multiple adjuvant clinical trials, tumor lymphocytic infiltration has been associated with favorable overall survival and long term outcomes. Measurement of TILs by Salgado Criteria has been validated retrospectively in numerous clinical trials, and the methodology has reached international consensus. The TILs prognostic marker is a continuous variable, with increases in TIL count associated with linear increases in distant disease free survival, independent of other prognostic markers such as lymph node status.

The rationale for measuring TILs by Salgado criteria is to evaluate the hypothesis that IRX-2 increases TIL count in early stage breast cancer. If so, then these data will support further study of the IRX-2 regimen in early stage breast cancer in a larger clinical trial with a clinical endpoint.

In cohort B, pathologic complete response rate will be assessed preliminarily in the setting of IRX-2 pre-treatment, on the basis that enhanced immune infiltration may mediate clearance of chemotherapy-resistant tumor cells.

3.1.3 Rationale for Exploratory Objectives

Additional immune-based biomarkers, while less extensively validated than H&E TIL count, hold promise as prognostic/predictive indicators, and may also be used to further characterize the immunologic and tumor effects of immunotherapy [Tumeh, 2014; Feng, 2015]. It is also quite possible that mechanistic insights may be obtained from such analyses. For example, if IRX-2 is shown to increase PD-L1 expression on TILs, this would support a follow-up pre-clinical assessment of combination IRX-2 with PD-1/L1 blockade.

3.1.4 Benefit/Risk Assessment

This trial is designed to incorporate immunotherapy into the early stage breast cancer paradigm while minimizing deviations from standard peri-operative care. The trial will only enroll subjects for whom standard neoadjuvant systemic therapy is not planned. At Providence Health Centers, among early stage patients not treated with neoadjuvant systemic therapy, the median time from diagnosis (by core biopsy) to surgical resection between 2004 and 2015 was 27 days (interquartile range 19-39 days, n=5165) [Page, unpublished observations]. Therefore, subjects enrolled in this trial are not anticipated to experience a delay in surgery beyond what is customary for the institution. To minimize delays in definitive surgical resection, subjects will be screened for eligibility and consented by the investigator as soon as possible following confirmation of invasive carcinoma. The study regimen calls for 21 days of pre-operative therapy, and therefore if subjects are consented and commence therapy within 7-10 days of diagnosis, they will be scheduled to undergo definitive surgical resection between days 23 and 28 after diagnosis, consistent with the median time from diagnosis to surgical resection among early stage subjects not treated with neoadjuvant systemic therapy.

This regimen has not raised any significant safety concerns in several phase I/II clinical trials as discussed above, and therefore the risk of significant harm from adverse events is minimal. Furthermore, the IRX-2 regimen has been administered pre-operatively in HNSCC patients, i.e., in patients who undergo more extensive surgical procedures than breast cancer patients, including elective neck dissection and more complicated tumor excisions and reconstructive surgery.

One potential indirect risk is that the study drugs might influence prognostic markers obtained from the surgical specimen, for example RNA expression profiling for ER-positive tumors used to determine chemotherapy benefit. For this reason, in all subjects with ER-positive breast cancer, a portion of the pre-study diagnostic core biopsy will be submitted for RNA expression profiling if clinically indicated.

Additional support for a low risk from the pre-operative IRX-2 regimen is that in a recent peri-operative early stage breast cancer immunotherapy trial, subjects were treated with either ipilimumab single dose, tumor cryoablation, or both. The regimen was well-tolerated, with no sustained adverse events, no delays in pre-planned surgical resection, and no reported deviations/complications in post-mastectomy adjuvant chemotherapy/hormonal therapy (Page, 2014).

3.2 Selection of Study Population

3.2.1 Inclusion Criteria

To be enrolled in the study, subjects must meet the following inclusion criteria.

1. Invasive breast cancer of any receptor subtype diagnosed by core-needle biopsy
2. To undergo surgical resection with curative intent by partial mastectomy (lumpectomy) or mastectomy
3. Tumor >5 mm in maximum diameter by ultrasound or mammography. (Subjects with smaller tumors may be included at the discretion of the Principal Investigator.) Cohort B: triple negative (defined by ER<10%, PR<10%, and HER2-negative by NCCN guidelines), T1c+ tumors for which neoadjuvant anthracycline-based and non-platinum containing chemotherapy is planned.
4. Willing and able to provide written informed consent, including consent for use of available tissue and required blood draws for research purposes
5. Availability of at least one tumor-bearing core specimen from the breast cancer diagnostic biopsy
6. Performance status of KPS 70% or greater
7. Female or male ≥18 years of age on day of signing informed consent
8. Adequate organ function, defined as:
    a. Absolute neutrophil count ≥1,500/μL.
    b. Platelet count ≥100,000/μL.
    c. Serum creatinine ≤1.5 × ULN or CrCl ≥60 mL/min (calculated by by Cockcroft-Gault Equation [Appendix 2]).
    d. AST and ALT ≤2.5 × ULN
    e. Bilirubin ≤1.5 × ULN or direct bilirubin ≤ULN. (Not applicable to patients with Gilbert's Syndrome.)
    f. INR/PT and PTT ≤1.5 × ULN
    g. Negative serum or urine pregnancy test for women of childbearing potential
9. Men, and women of childbearing potential must use acceptable measures to avoid becoming pregnant or a new father while on treatment and for 1 year after last dose of investigational therapy

3.2.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study.

1. Neoadjuvant systemic therapy is planned (except for Cohort B)
2. Prior surgery, radiotherapy or chemotherapy for this cancer (other than core-needle biopsy or lymph node excisional biopsy)

3. Received an investigational agent within 4 weeks of the first dose of treatment.
4. Diagnosis of immunodeficiency or has received more than replacement doses of corticosteroids any other immunosuppressive therapy within 4 weeks of the first dose of treatment
5. Hypersensitivity to IRX-2, cyclophosphamide, indomethacin, omeprazole, aspirin or ciprofloxacin.
6. Chronic anticoagulation, not including aspirin, but including heparins, warfarin, oral anticoagulants or other platelet function inhibitors , that cannot, in the documented opinion of the investigator, safely be interrupted from at least 2 days prior to the initiation of the study regimen until after surgical resection of the tumor.
7. Another malignancy that required active treatment within 6 months of the first dose of treatment
8. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, such that trial participation is not in the best interest of the subject, including but not limited to uncontrolled hypertension or clinically significant cardiovascular disease, myocardial infarction within the previous 3 months, active infection or pneumonitis or other pulmonary disease requiring systemic therapy, clinically significant gastritis or peptic ulcer disease (that would preclude the use of indomethacin), stroke of other symptoms of cerebral vascular insufficient within the last 3 months, autoimmune disease that has required systemic treatment within the past 2 years (other than hormone replacement doses), or uncontrolled psychiatric or substance abuse disorders.
9. Pregnancy or lactation.
10. Known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected.

3.3 Tumor Staging

Initial tumor staging will be performed using the TNM staging and stage grouping is based on American Joint Committee on Cancer (AJCC) clinical staging criteria:
https://cancerstaging.org/references-tools/quickreferences/Documents/BreastMedium.pdf

3.4 Assessment of Surgical Resectability

The disease must be resectable for cure, as determined by the investigating clinician (in consultation with a surgeon if necessary), in order for the subject to be eligible for the study.

3.5 Termination of Study Treatment

The investigator may stop the study treatment regimen due to an allergic or hypersensitivity reaction to the study drug, an SAE or clinically significant AE or laboratory abnormality or for protocol noncompliance. If a subject is discontinued from the study due to an AE, the investigator should notify IRX Therapeutics within 24 hours of the event.

NOTE: Subjects may experience changes in primary tumor or regional lymph nodes during treatment with the IRX-2 regimen, including inflammation, necrosis, or change in consistency. These changes should not be mistaken for tumor progression prior to surgery.

3.6 Withdrawal of Consent

Any subject may terminate participation in the study at any time but every effort should be made to continue with study treatment and evaluations. If a subject elects to discontinue study

participation at any time for safety, medical or personal reasons, the investigator should make a reasonable effort to determine the reason for the subject's withdrawal and document the reason on the CRF.

Any clinically significant AEs or SAEs leading to premature withdrawal are to be followed until resolution.

4 STUDY TREATMENTS

4.1 Study Design

Subjects who meet all eligibility criteria will be treated with the IRX-2 regimen as described in Table 1 above (Section 3.1).

4.2 Regimen Medications

4.2.1 IRX-2

IRX-2 is supplied as a pale yellow, sterile liquid for subcutaneous injection in 2.0 mL single-dose vials. All immediate study supply containers will be appropriately labeled to identify study number, kit numbers, lot number, and product identity. Kit and lot numbers of IRX-2 will be recorded in the CRF. Study syringes for subcutaneous IRX-2 administration will be disposed of in accordance with biosafety procedures.

4.2.2 IRX-2 Storage

IRX-2 must be stored in a secure area and maintained under labeled storage conditions at a range of −15°C to –25° C.

4.2.3 IRX-2 Administration

IRX-2 should be administered as two subcutaneous injections of 1 mL each, in the periareolar region of the affected breast. Each injection should be given subcutaneously approximately 1 cm from the outer margin of the areola. A 4-6 mm, 28-30 gauge needle is recommended; injections should consistently enter the fat just underneath the skin. The first injection should be administered on the same axis as the tumor location as identified by physical exam, mammography or ultrasound. The second injection on the same day should be rotated 90 degrees clockwise to the first injection. On the second day of IRX-2 injections, the first injection should be again administered on the same axis as the tumor location, but the second injection should be rotated 90 degrees counter-clockwise to the first injection, i.e. to maximize the likelihood of drug delivery to the lymphatics, the location of the second injection each day should be alternated between clockwise and counter-clockwise from the primary injection site. Each injection site each day should be gently massaged for one minute to promote entry into lymphatics.

All IRX-2 injections will be administered by study personnel at the study site.

4.2.4 Other Medications

Commercial cyclophosphamide will be provided by the pharmacy of the study site. The relatively low dose of cyclophosphamide has low to moderate emetic risk per NCCN Guidelines. Use of corticosteroids as an antiemetic is neither required nor permitted because of its potential immunosuppressive effect. An antiemetic regimen of lorazepam and a serotonin (5-HT3) antagonist is recommended.

IRX Therapeutics will supply indomethacin, zinc (with multivitamins) and omeprazole.

4.3 Method of Assigning Subject Numbers

After a subject has signed the informed consent form and has been deemed eligible for enrollment, the investigator will assign a unique subject number to that subject. Subjects who withdraw from the study after being assigned a subject number will retain that number.

4.4 Treatment Compliance

The investigator or study personnel will administer IRX-2 injections as described in Section 4.2.4. Subjects must maintain a log of all oral medications taken and this must be documented in the clinical record.

4.5 Prohibited, Prior and Concomitant Treatments

Subjects should **not** take aspirin (except for low-dose aspirin as prescribed for vascular disease or cardiac risk-reduction) or other non-prescribed, non-steroidal anti-inflammatory agents.

All other concomitant prescribed medications, recognized over-the-counter medications, or any changes in medications during the study from Day 1 to the day of surgery will be collected. Thereafter, only medications given to treat a reported study related AE or SAE will be collected.

5 STUDY PROCEDURES AND SCHEDULE

5.1 Study Schedule

The Schedule of Study Procedures is outlined in Table 2.

Tests and procedures should be performed on days indicated. Variation of ±3 days for tests indicated in “days”, 1 week for tests indicated in “weeks” is permitted.

Table 2: Schedule of Study Events

Study Procedure	Screening[a]	Treatment Period[b]			Follow-Up		
					Post treatment[b]	Surgery/ Biopsy[b, c, k]	Post-Surgery/ Biopsy
Study Day	**-21 to -1**	**Day 1**	**Day 1-21**	**Any 10 days between Day 4-19**	**~Day 18-27**	**Within 12 days of last IRX-2**	**~30 days post**
Informed consent	X						
Inclusion/Exclusion	X						
Study Drug Administration							
Cyclophosphamide		X[d]					
Indomethacin, zinc with multivitamins			X[e]				
Omeprazole			X				
IRX-2				X[f]			
Evaluations							
Complete history & physical examination, including ECOG	X						
Interval history & physical examination of primary site and regional nodes					X		X
Adverse events, concomitant medications[g,h,]		X	X	X	X		X
CBC, chemistry panel[i, j]	X	X	X		X		X
PT, PTT[i]	X						
Pregnancy test, if applicable	X						
Core biopsy (archival or new biopsy)	X						
Path specimen for research						X	
Blood and/or serum samples[j]		X[j]	X[j]		X[j]		X[j]

Footnotes for Schedule of Events Table:

a. Subjects may be screened for eligibility up to 21 days prior to Day 1.
b. As necessitated by scheduling logistics, the oral medications may be discontinued after Day 15 and surgery performed 2 days later.
c. Surgery (or biopsy for cohort B) is ideally to be scheduled 2-5 days following the last IRX-2 injections, but may be within 12 days of the last IRX-2 injection.
d. Corticosteroids should not be needed as a prophylaxis because cyclophosphamide dose is associated with minimal nausea. Suggested antiemetic regimen would include a serotonin (5-HT3) antagonist and lorazepam.
e. Indomethacin must be discontinued at least 2 days before surgery.
f. Two 1 mL subcutaneous periareolar IRX-2 injections may be given on any 10 days between Days 4 and 17 (days 4-8 and 11-15 preferred).
g. Adverse events will be measured from Day 1 until the day of surgery. Adverse events must be recorded as observed, but at least on Day 1 (before cyclophosphamide), Day 4 (before and after first IRX-2 injections), and twice on Days 9-17 (after approximately 5 and 10 days of IRX-2 injections).
h. All concomitant medications should be documented at baseline. Thereafter, only medications given to treat a reported AE or SAE, or considered immunosuppressive or anti-proliferative should be documented.
i. Day 1 CBC and chemistry panel will not be performed if Screening / Baseline visit is less than 7 days prior. PT/PTT at screening only.
j. Three 10 cc CPT tubes and one 5cc Cytochex tube for research studies (including immunogenicity testing) will be collected on Day 1 (before cyclophosphamide), ~Day 4 (before first IRX-2 injections), twice on Days 9-19 (after approximately 5 and 10 days of IRX-2 injections), prior to surgery and at the post-operative visit. In addition, 5 mL of blood will be collected at -, on approximately Day 17 and on the post-operative visit for serum analysis of immunogenicity testing against the active components of IRX-2. CBC/diff and chemistry: CMP, LDH will be drawn at the same time points.
k. Cohort B will undergo research core biopsy during this time, and will commence neoadjuvant chemotherapy within 7 days of biopsy

5.2 Study Procedures

5.2.1 Screening

The purpose and procedures of the study will be fully explained to participants. Those wishing to enroll in the study will sign a written informed consent prior to initiating any protocol specific evaluations or procedures (unless performed within study window prior to signing consent as part of standard-of-care visits or procedures)..

The following screening evaluations are to be performed within 21 days (unless otherwise noted) prior to Day 1:

- Medical history, including assessment of all entry and exclusionary criteria, demographics (age, sex, race, menstrual status, weight, height), history of present illness, history of hypersensitivity (drug allergies), general medical history and habits.
- Determination of ECOG performance status (Appendix 1).
- Concomitant medication review.
- Physical exam, including description of the breast and regional lymph node areas.
- Clinical laboratory assessments:
    - Hematology: complete blood count (CBC), differential, platelet count, PT and PTT.
    - Serum chemistry: serum albumin, total protein, serum bilirubin, lactate dehydrogenase (LDH), ALT/SGPT, AST/SGOT, alkaline phosphatase, creatinine, blood urea nitrogen (BUN), glucose, and electrolytes.
        - Calculated creatinine clearance (if creatinine >1.5 × ULN) (by Cockcroft-Gault Equation [Appendix 2]).
- Pregnancy test (serum or urine) if appropriate.
- Tumor biopsy for confirmation of diagnosis and research specimen (unless diagnostic biopsy confirmed sufficient by pathology).

5.2.2 Day 1

The following procedures will be completed at this visit:

- Collection of research blood and serum samples
- Administer the following medications:
    - Infusion of cyclophosphamide, 300 mg/m^2. Steroids must not be administered as antiemetic agents since these might interfere with immunomodulation by IRX-2. Antiemetic regimen should include a serotonin (5-HT3) antagonist and lorazepam or equivalent medications.
    - Begin indomethacin (25 mg, TID) to be taken orally after meals from Day 1 through Day 21.
    - Begin zinc (15-30 mg, once daily) with multivitamins to be taken orally from Day 1 through Day 21.
    - Begin omeprazole (20 mg, once daily) to be taken orally after meals from Day 1 through Day 21.

5.2.3 Any 10 days between days 4 and 19

- IRX-2 should be administered by subcutaneous injection at two sites as described in Section 4.2.3.
- Each subcutaneous injection will be approximately 1.0 mL of IRX-2 (corresponding to 115 units of IRX-2 per injection or 2300 units during the total regimen).
- On the first day of IRX-2 injections, subjects will be monitored for 15 minutes after the IRX-2 injections for signs or symptoms of any reaction.

If the nurse or other qualified medical personnel receives information about an AE, she/he will document the event.

In the event of Grade 3 or higher toxicity, no further IRX-2 should be injected and indomethacin should be discontinued. In the event of Grade 2 toxicity, treatment should be delayed until stable and then resumed per protocol. Treatment should be discontinued in a subject if the treatment is delayed for more than 7 days, except that treatment with IRX-2 and zinc with multivitamins may be continued per protocol in a subject intolerant of indomethacin.

5.2.4 One to Five Days Prior to Surgery (or post-IRX2 biopsy)

The following procedures will be performed at this visit:

- Interval history and assessment of all current symptoms including severity (AEs).
- Record concomitant medications.
- ECOG performance status.
- Brief physical exam.
- Breast and regional node exam.
- Indomethacin must be stopped at least 2 days before surgery/biopsy. Subjects are scheduled to take indomethacin through Day 21. If surgery/biopsy is to be performed before Day 21, then the subject should discontinue indomethacin at least 2 days before surgery.
- Clinical laboratory assessments:
    - Hematology: CBC, differential, platelet count.
    - Serum chemistry: serum albumin, total protein, serum bilirubin, LDH, ALT/SGPT, AST/SGOT, alkaline phosphatase, creatinine, BUN, glucose, and electrolytes.
- Collect blood samples for correlative studies.

5.2.5 Surgery (or post-IRX biopsy)

All early stage breast cancer subjects will undergo surgical resection of their tumor, reconstruction, and postoperative adjuvant therapy per usual standards of care. Surgery is ideally to be scheduled 2-5 days following the last IRX-2 injections, but may be within 12 days of the last IRX-2 injections. On the rare occasion, subjects/investigators may opt for post-IRX-2 biopsy during this window instead of surgical resection, i.e. if original surgical date must be revised to accommodate unforeseen changes in surgical plan unrelated to the IRX-2 treatments. Subjects in Cohort B will undergo core biopsy instead of surgery, but will undergo standard-of-care surgical resection following completion of neoadjuvant chemotherapy, or may advance to surgery at any time at the discretion of the investigator.

- For all subjects, surgical and pathological data must be reported as described in this Protocol and the CRFs.

5.2.6 Follow-up Schedule

Subjects will be assessed clinically at a final study visit approximately 30 days following surgical resection. Efforts will be made for this visit to coincide with a standard-of-care clinical assessment.

Subjects will be followed longitudinally, in an ongoing basis, to evaluate for post-IRX recurrence free survival. Because recurrences may occur many years later, no pre-defined termination date for follow-up is designated.

5.2.7 Cohort B

Subjects in Cohort B (i.e. those with triple negative breast cancer receiving post-IRX-2 neoadjuvant chemotherapy) will receive standard of care neoadjuvant anthracycline-based chemotherapy. Subjects for whom platinum-based neoadjuvant therapy is planned are not eligible to enroll. Pathologic response rate in the surgical resection specimen will be assessed following chemotherapy. Remaining tissue from the resection specimen, in excess of tissue needed for diagnostic purposes, may be used to conduct additional immunologic exploratory assessments.

Subjects in cohort B will follow the same toxicity and immunologic assessments and long-term follow-up as cohort A, including a 30-day post-biopsy safety visit. Following the 30-day window, toxicity assessments pertaining to chemotherapy will not be recorded formally in the trial, but will be conducted per standard-of-care at the discretion of the treating physician.

6 ADVERSE EVENTS AND REPORTING REQUIREMENTS

6.1 Adverse Event Definitions

Definitions of AEs will follow the International Conference on Harmonization E6: Good Clinical Practice Step 5, Consolidated Guideline 1.5.96, April 1998 edition, and are summarized below. For this study, the investigational product is the IRX-2 regimen, including IRX-2, cyclophosphamide, indomethacin, and zinc-containing multivitamins.

An adverse drug experience (event) is any new undesirable medical occurrence or change (worsening) of an existing condition in a subject that occurs from Day 1 until the time of surgery (with the exception of an SAE, if it occurs within 30 days from the last dose of study medication), whether or not considered to be drug related. Therefore, AEs are treatment-emergent signs and symptoms. This includes those events occurring from drug overdose, whether accidental or intentional, from drug abuse or from drug withdrawal. In general, abnormal laboratory findings without clinical significance based on the investigator's judgment are part of the database and should not be recorded as AEs.

Adverse events reported by the subject or observed by the investigator will be individually listed on an AE CRF page. The signs and symptoms, date of onset, duration, action taken, severity, outcome to date, and relationship to the study drug will be recorded. The severity of AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.03 toxicity criteria. Severity will be classified as described in Section 6.1.3.

For adverse events of Grade 3 or 4, the Principal Investigator will determine whether to modify or discontinue study treatment.

Adverse events will be measured from Day 1 until surgery and then 30 days post-surgery. Evidence of toxicity after surgery will be assessed by tabulation of AEs felt by the investigator to be definitely or possibly related to the study regimen.

Serious adverse events will be measured from Day 1 until surgery and then at 30 days post-surgery. Evidence of toxicity after surgery will be assessed by tabulation of SAEs felt by the investigator to be definitely or possibly related to the study regimen. Ongoing AEs at the time of surgery will be followed for 30 days post-surgery. Ongoing SAEs beginning from surgery will be reported and monitored until resolution or stability.

At each visit, the investigator will be prompted to report AEs as “not”, “possibly,” or “definitely” related to the IRX-2 regimen.

6.1.1 Definition of Serious and Life Threatening Adverse Events

A serious adverse drug experience (event) is any AE occurring at any dose that results in any of the following outcomes:

- Death
- Is life-threatening
    - A life-threatening adverse drug experience is any AE that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This does not include a reaction that, had it occurred in a more severe form, might have caused death.
- A persistent or significant disability/incapacity
- Inpatient hospitalization or prolongation of existing hospitalization, except that planned hospitalization during days in clinic and surgery or hospitalization for the social reasons or the convenience of the subject or physician shall not be considered an SAE
- A congenital anomaly/birth defect
- Important medical event
    - Important medical events that may not result in death, be life-threatening, or require hospitalization may be serious when, based upon appropriate medical judgment, the event may jeopardize the subject and require medical or surgical intervention to prevent one of the outcomes listed above. Severe complications of standard-of-care surgical resection (lumpectomy or mastectomy), are to be considered “important medical events” and thus SAEs.

6.1.2 Definition of Relationship to Study Regimen and Drug

Association or relatedness to the study regimen will be graded as either “not”, “possibly,” or “definitely” related to the study regimen. Determination of relatedness includes:

- **Definitely**, characterized as an AE that
    - Follows direct temporal sequence from regimen administration.
    - Abates upon discontinuation of the regimen.

- Cannot be explained by the known characteristics of the subject's clinical state or by other modes of therapy administered to the subject.

- **Possibly**, characterized as an AE that
  - Follows a reasonable temporal sequence from regimen administration.
  - Abates upon discontinuation of the regimen.
  - Could have been produced by the subject's clinical state or by other modes of therapy administered to the subject.
- **Not Related**, characterized as an AE that
  - Does not follow any temporal sequence from regimen administration.
  - Is explained by the subject's clinical state or by other modes of therapy administered to the subject.

6.1.3 Definition of Severity

The severity of adverse changes in physical signs or symptoms will be graded according to the NCI-CTCAE v4.03. For AEs not listed in the table, the severity of adverse changes in physical signs or symptoms will be classified as follows:

Grade 1: **Mild** (transient or mild discomfort; no limitation in activity; no medical intervention/therapy required)

Grade 2: **Moderate** (mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required)

Grade 3: **Severe** (marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible)

Grade 4: **Life-threatening** (extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable)

Grade 5: **Death**

6.1.4 Definition of Action Taken

None: No action taken with regard to the study drug

Interrupted: The study drug was stopped but restarted after the subject's symptom abated. The subject was rechallenged with the study drug

Discontinued: The study drug was permanently stopped

6.1.5 Definition of Outcome to Date

Resolved with sequelae: The subject has recovered from the AE with observable residual effects

Resolved without sequelae:	The subject has fully recovered from the AE with no observable residual effects
Unresolved:	The AE is present and observable
Death:	The subject died as a result of the AE
Lost to Follow-up:	Source documentation confirms that repeated attempts to contact subject have failed

6.2 Notification of Serious or Unexpected Adverse Events

The Principal Investigator is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32). A suspected adverse reaction must be both serious and unexpected in order to meet the reporting requirements.

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or IRX Therapeutics, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator’s initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

Providence Portland Medical Center will also notify IRX of all SAEs in parallel with notification to the FDA.

7 STATISTICAL CONSIDERATIONS AND ASSESSMENT OF ENDPOINTS

7.1 Sample Size Considerations

This clinical trial is a proof of concept and initial tolerability/safety study but not a “first-in-human” study. The number of subjects is generally consistent with similar studies of other investigation products at this stage of development. A sample size of 20 was selected because it provides adequate power to estimate the proportion of subjects who tolerate the regimen, and to preliminarily characterize the immunologic effects of therapy.

Cohort B expansion is designed similarly to evaluate feasibility and immunologic effects of pre-neoadjuvant IRX-2 in a higher-risk triple negative breast cancer population. Cohort B sample size of n=19 is also based upon the first stage of a Simon 2-stage design, to test the hypothesis that IRX-2 pre-treatment improves pathologic complete response rate to 50% compared to historical benchmark of 30% (based upon a meta-analysis of non-platinum containing chemotherapies).

Subjects who are enrolled but withdraw consent prior to beginning the IRX-2 Regimen will not be evaluable and will be replaced.

7.2 Primary Endpoint: Safety and Tolerability of the IRX-2 Regimen (cohort A)

Definitions and reporting requirements for AEs and SAEs have been presented above in Section 6.

Because this therapy is being administered in subjects receiving therapy intended to cure their disease, the threshold for establishing safety/tolerability will be high, such that nearly all subjects must tolerate the therapy. The therapy will be deemed “safe and tolerable” if 75% or greater of evaluable subjects tolerate the therapy according pre-specified definition of tolerability.

This is based upon hypothesis testing whereby the null hypothesis is that the regimen is not tolerable (i.e. falls below the boundary of tolerability), and the alternative hypothesis is that the regimen is tolerated. The boundary of tolerability will depend on the total number of evaluable subjects, as depicted in the below table.

For example, using the 75% threshold of tolerability and assuming that 20 subjects are evaluable, if 15 or more out of 20 patients tolerate the therapy according pre-specified definition of the tolerability (i.e., if the tolerability rate is 75% or greater), then the null hypothesis H_0: $p <= 0.58$ (i.e., the tolerability boundary) would be rejected in favor of alternative hypothesis H_1: $p = 0.8$ with a type I error rate and power equal to 0.09 and 0.80, respectively.

# *evaluable subjects (n)*	*75% threshold (x*)*	x^* / n	*Tolerability boundary under* H_0	*Specified Tolerability under* H_1	Type 1 error	Power
15	12	0.80	0.60	0.85	0.09	0.82
16	12	0.75	0.56	0.81	0.10	0.83
17	13	0.76	0.58	0.82	0.09	0.82
18	14	0.78	0.60	0.83	0.09	0.82
19	15	0.79	0.62	0.84	0.10	0.82
20	15	0.75	0.58	0.80	0.09	0.80

Subjects will be considered evaluable if they consent and receive any part of the cyclophosphamide infusion. The rate of Grade 1-4 toxicities will be reported as additional, descriptive measures of tolerability. The following criteria must be met for that subject to be considered to have "tolerated" the therapy:

- Subject receives cyclophosphamide, >7 injections of IRX-2 and >10 days of indomethacin and multivitamins with zinc and does not have Grade 3 or 4 treatment-associated adverse events requiring discontinuation of therapy;
- Subject does not experience a delay in standard-of-care surgical resection related to treatment-associated adverse events;
- Subject does not have any ongoing treatment-related Grade 2-4 adverse events at the 30-day post-operative follow-up visit;
- Subject does not withdraw consent after the start of the cyclophosphamide infusion.

7.2.1 Laboratory values, vital signs, physical findings and other safety data

Any laboratory results, vital signs, physical findings, or outcomes of any other investigations that are conducted during the course of the study that in the opinion of the investigator should be considered an adverse event must be documented in the appropriate section of the CRF.

7.2.2 Pregnancy

All pregnancies that occur during the study or for which conception is likely to have occurred within 1 year of the last administration of IRX-2 must be reported as an adverse event, including pregnancies that occur in the partner of a subject.

All pregnancies are to be monitored for the duration and outcomes reported. Congenital anomaly or birth defects should be reported as an SAE.

7.3 Secondary Endpoint: Stromal TIL infiltration

The secondary endpoint (cohort A) is to evaluate the effect of therapy on stromal TIL (sTIL) infiltration by Salgado criteria. The rationale for this endpoint is that the percentage of TILs in pre-operative biopsies is highly correlated with both overall survival and response to neoadjuvant therapy. If the IRX-2 regimen is found to increase the percentage of TILs, this would provide rationale to evaluate further IRX-2 in this setting.

Lymphocyte infiltrates in the pre-treatment tumor biopsies and the surgical specimens will be determined and compared. We assume that a mean sTIL count is 10% and standard deviation is 17% (adopted from a recent meta-analysis, Loi et al 2015). Since the data will be observed at pre and post times on each tumor specimen, we also assume that those pairs of pre and post measurements are equally correlated with a correlation coefficient of at least 0.1. A sample size of n=20 was determined to be sufficient to attain a power of 0.80, associated with a one-sided hypothesis test given type I error rate 0.1, to detect a mean increase in TIL score of 11% following receipt of IRX-2.

7.4 Exploratory Endpoints

Exploratory endpoints will be analyzed descriptively, and will include additional peripheral blood and intratumoral analyses of the immunologic effects of the IRX-2 regimen, including, for example, some or all of the following:

- flow cytometric quantification and characterization of peripheral lymphocytes (including activated T-cells, T-regulatory cells, NK cells, and myeloid cells)
- TIL phenotype characterization by multispectral immunofluorescence imaging to assess changes in abundance of T-regulatory cells, activated T-cells, myeloid lineages and dendritic cells. Subsets of lymphocytes will be evaluated including total T cells and naïve, cytotoxic and regulatory T cells. The analyses may include quantifications of cellular infiltrates and degree of activation by immunohistochemistry including (but not limited to): CD3, CD8, CD68 (myeloid derived suppressor cells), CD45RO and Fox P3 on lymphocytes and MHC class I and II and PDL1 on tumor cells. Other immune subsets and activation markers may be evaluated.
- Intratumoral and T-cell clonal responses by T-cell receptor DNA deep sequencing
- Characterization of intratumoral immune responses by RNA expression using the Nanostring PanCancer Immune panel and/or Prosigna tumor recurrence score

Exploratory endpoints will be determined in all subjects enrolled but some of these endpoints may be modified during the course of the study, so long as no additional blood or tissue specimens are required of study subjects.

7.5 Cohort B Statistical Considerations

Cohort B will also test the feasibility and immune effects of IRX-2, however in a pre-neoadjuvant chemotherapy setting. Secondarily, the cohort is designed to be expanded (per a subsequent protocol revision) if a threshold pCR rate is attained, allowing for a formal comparison of pCR against a historical benchmark. We have selected a benchmark pCR rate of 30%, based upon a recent meta-analysis of five randomized trials that evaluated non-platinum containing neoadjuvant chemotherapy. (Petrelli F, et al). In a minimax design, we will test the null hypothesis of 30% pCR rate, versus an alternative hypothesis of 50% pCR rate, with alpha of 0.05 and beta of 0.2. With this design, response rate of ≥7/19 will prompt consideration of an expansion to total 39 patients. Responses in ≥17/39 would prompt the team to reject the null hypothesis, and consider the regimen meritorious for further study. With this design, the probability of early stopping is 0.67. Pathologic complete response will be assessed by an intention-to-treat principle, whereby all subjects receiving at least one partial dose of chemotherapy will be considered evaluable. Subjects lost to follow-up or not evaluable will be considered non-pCR. Pathologic complete response will be assessed by local pathologist, defined by the AJCC staging system (ypT0/Tis ypN0). Subjects who do not receive at least one partial dose of chemotherapy will be replaced.

8 ETHICAL AND REGULATORY CONSIDERATIONS

8.1 Protection of Human Subjects

This study will be conducted in accordance with the US CFR, Title 21, Parts 11, 50, 54, 56 and 312; the Declaration of Helsinki (1964) including all amendments and revisions; the Good Clinical Practices: Consolidated Guideline (E6), International Conference on Harmonization, the Food and Drug Administration Guidance for Industry: Computerized Systems Used in Clinical Trials, the ethical principles that have their origins in the Declaration of Helsinki and all applicable country-specific and local regulations. All required study information will be archived as required by regulatory authorities.

8.2 Informed Consent

Informed consent must be obtained and documented prior to the initiation of non-standard of care screening tests, study registration and study therapy.

The investigator (or designee) will explain the study procedures, treatments, costs, potential benefits/risks involved, and the alternatives for care or treatment to the patient/legally authorized representative (hereafter referred to as “patient”). Determination of the appropriate person to give legal consent will be done in accordance with ICH/GCP, Providence Health & Services Policy and state law. All study procedures will be explained in terms the patient can understand and an opportunity will be given for the patient to ask questions. Special care will be given to explaining the use and disclosure of a patient’s protected health information and if applicable, a patient’s rights if the study involves genetic research. This must be done before the informed consent is signed. This discussion will be documented in the patient’s chart.

A copy of the signed consent form will be provided to the patient and the original consent form will be kept in the patient chart at the Clinical Trials Office.

9 STUDY ADMINISTRATION

9.1 Monitoring Plan

9.1.1 Site Initiation

The Principal Investigator and Research Nurse will conduct an implementation meeting for sub-investigators and applicable research and clinic staff. Attendance at this meeting will be documented. Anyone unable to attend must complete an online study training in HealthStream prior to conducting any study-related activities.

9.1.2 Interim Monitoring

Interim monitoring will be scheduled at regular intervals throughout the study, with the timing being dependent on subject enrollment. Generally, the study monitor will compare the data entered on the CRFs with the hospital or clinic records (primary source documents) and check for protocol compliance, including verification of informed consent, all subject visit dates, all AEs, all concomitant medications, and all key efficacy observations. In addition, the Site Regulatory Binder containing essential documents, study drug accountability, and supporting records will be reviewed. Findings from this review will be summarized in writing and presented to the investigator, and the dates of the monitoring will be recorded by the study monitor in a sign-in log to be kept in the Site Regulatory Binder.

9.1.3 Study Close-out

At the study's conclusion close-out monitoring will be performed, including a final review and reconciliation of all study and regulatory documents.

9.1.4 Site Responsibility

The procedures defined in the protocol and in the CRFs will be carefully reviewed by the investigator and his/her staff, prior to the time of study initiation, to ensure appropriate interpretation and implementation. No deviations from the protocol should be made. Minor exceptions may be approved on a case-by-case basis and must be authorized by the Principal Investigator and documented in writing. Significant deviations that may impact subject safety or study integrity will be reported to the IRB and may result in termination of study participation. Any changes to the protocol will originate from the Principal Investigator in the form of an amendment.

9.2 Investigational Product Accountability

Drug accountability records will be maintained by the Providence Health & Services Oregon Region Investigational Drug Services pharmacy. Drug accountability records will be initiated with pharmacy receipt of drug. An Investigational Drug Accountability log will be used to account for all investigational drugs. At the time of dispensing, the IDS pharmacist or technician will log appropriate information regarding the agent being dispensed, including but not limited to: name of drug, strength, quantity dispensed, kit/vial/lot number, subject ID number, initials and date. At the time of dispensing, the DARF will be reconciled with the physical inventory.

Used vials of investigational drug will be destroyed as per PH&S – Portland Service Area – EOC Policy on Waste Management, EC 315:
http://in.providence.org/Documentscentral/OR/safety/Waste%20Management%20Disposal.pdf

Unused investigational drug will be returned or destroyed at study close or when drug expires as per the referenced policy above.

All investigational drug accountability records, physical inventory and other supporting documents (such as temperature monitoring logs) will be made available to the monitor and applicable regulatory agencies for auditing and monitoring purposes.

9.3 Study Documents and Access to Records

It is the responsibility of the investigator and study staff to maintain a comprehensive and centralized filing system of all study-related documentation, which is suitable for inspection at any time by the study monitor, the Sponsor or its designee, the FDA and other Regulatory Agencies. Elements should include, but are not limited to:

- Study Files, containing the curriculum vitae of all investigators and his/her designees, FDA Form 1572, the protocol with all amendments, the IB, local lab certifications and lab normal ranges, and all correspondence to and from local Regulatory Authorities.
- Subject Files, containing the completed original supporting source documentation and the signed informed consent form(s).
- Dispensing records of test agents.
- In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is

not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

9.4 Quality Assurance

The Principal Investigator and all designees will ensure the integrity of all data collected and calculations made during the conduct of the study according to their quality assurance standards of operations. Edit checks will be run on the data and queries issued. All data will undergo 100% review.

9.5 Confidentiality and Publication Policy

This trial will be listed in clinical trial databases as appropriate, e.g., www.clinicaltrials.gov.

10 REFERENCES

Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN et al. Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014. doi:10.1200/JCO.2013.55.0491.

American Cancer Society. Breast Cancer Facts & Figures 2011-2012. Atlanta: American Cancer Society, Inc. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-030975.pdf.

Berd D, Mastrangelo MJ, Engstrom PF, et al. Augmentation of the human immune response by cyclophosphamide. Cancer Res 1982;42:4862-4866.

Berinstein NL, Wolf GT, Naylor PH, et al. Increased lymphocyte infiltration in patients with head and neck cancer treated with the IRX-2 immunotherapy regimen. Cancer Immunol Immunother 2012;61:771-782.

Bianchini C, Ciorba A, Stomeo F, et al. Immunonutrition in head and neck cancer: have a look before surgery! Eur Arch Otorhinolaryngol. 2012;Jan;269(1):5-8

Blewett HJ, Taylor CG. Dietary zinc deficiency in rodents: effects on T-cell development, maturation and phenotypes. Nutrients 2012;4:449-66

Brookes GB, Clifford P. Nutritional status and general immune competence in patients with head and neck cancer. J Royal Soc Med 1981;74:132-139.

Czystowska M, Han J, Szczepanski MJ, et al. IRX-2, a novel immunotherapeutic, protects human T cells from tumor-induced cell death. Cell Death Differ. 2009;16:708-718.

Czystowska M, Szczepanski MJ, Szajnik M, et al. Mechanisms of T-cell protection from death by IRX-2: a new immunotherapeutic. Cancer Immunol Immunother. 2011;60:495-506.

DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007;9(4):212.

Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105-13.

Egan JE, Quadrini KJ, Santiago-Schwarz F, et al. IRX-2, a novel in vivo immunotherapeutic, induces maturation and activation of human dendritic cells in vitro. J Immunother 2007;30:624-633.

Emens LA, Jaffee EM. Leveraging the activity of tumor vaccines with cytotoxic chemotherapy. Cancer Res 2005;65:8059-8064.

Feng Z, Puri S, Moudgil T, Wood W, Hoyt CC, Wang C et al. Multispectral imaging of formalin-fixed tissue predicts ability to generate tumor-infiltrating lymphocytes from melanoma. Journal for immunotherapy of cancer. 2015;3:47.

Freeman SM, Franco JLB, Kenady DE, Baltzer L, Roth Z, Brandwein HJ, Hadden JW. A Phase 1 safety study of an IRX-2 regimen in patients with squamous cell carcinoma of the head and neck. Am J Clin Oncol 2010;34:173-8.

Freiser ME, Serafini P, Weed DT. The immune system and head and neck squamous cell carcinoma: from carcinogenesis to new therapeutic opportunities. Immunol Res. 2013;57:52-59.

Gildener-Leapman N, Ferris RL, Bauman JE. Promising systemic immunotherapies in head and neck squamous cell carcinoma. Oral Oncol. 2013;49:1089-1096.

Good RA, Fernandes G. Nutrition, immunity, and cancer-a review. Part I: Influence of protein or protein-calorie malnutrition and zinc deficiency on immunity. Clin Bull 1979;1:3-12.

Haase H, Rink L. Zinc signals and immune function. Biofactors 2014;40:27-40

Hadden JW, Endicott J, Baekey P, et al. Interleukins and contrasuppression induce immune regression of head and neck cancer. Arch Otolaryngol Head Neck Surg. 1994;120:395-403.

Keen CL, Gershwin ME. Zinc deficiency and immune function. Ann Rev Nutr 1990;10:413-415.

Kuo WH, Chang YY, Lai LC, Tsai MH, Hsiao CK, Chang KJ et al. Molecular characteristics and metastasis predictor genes of triple-negative breast cancer: a clinical study of triple-negative breast carcinomas. PLoS One. 2012;7(9):e45831. doi:10.1371/journal.pone.0045831.

Lapointe H, Lampe H, Banerjee D. Head and neck squamous cell carcinoma cell line-induced suppression of in vitro lymphocyte proliferative responses. Otolaryngol Head Neck Surg 1992;106:149-158.

Loi S, Drubay D, Adams S, Francis PA, Joensuu H, Dieci MV et al., editors. Pooled individual patient data analysis of tumor infiltrating lymphocytes (TILs) in primary triple negative breast cancer (TNBC) treated with anthracycline-based chemotherapy. San Antonio Breast Cancer Symposium; 2015 12/18/2015; San Antonio, TX.

Machiels J-PH, Reilly RT, Emens LA, et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. Cancer Research 2001;61:3689-3697.

McMasters KM, Wong SL, Martin RC, 2nd, Chao C, Tuttle TM, Noyes RD et al. Dermal injection of radioactive colloid is superior to peritumoral injection for breast cancer sentinel lymph node biopsy: results of a multiinstitutional study. Ann Surg. 2001;233(5):676-87.

Miyashita M, Sasano H, Tamaki K, Chan M, Hirakawa H, Suzuki A et al. Tumor-infiltrating CD8+ and FOXP3+ lymphocytes in triple-negative breast cancer: its correlation with pathological complete response to neoadjuvant chemotherapy. Breast Cancer Res Treat. 2014;148(3):525-34.

National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, May 28, 2009.

National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer, Version I.2016.

Naylor PH, Hernandez KE, Nixon AE, et al. IRX-2 increases the T cell-specific immune response to protein/peptide vaccines. Vaccine 2010;28:7054-7062.

North RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 1982;55:1063-1074.

North RJ. The murine antitumor immune response and its therapeutic manipulation. Adv Immunol 1984;35:89-155.

Page D, Yuan J, Ginsberg A, editors. Tumor and systemic immune responses to pre-operative (pre-op) cryoablation (cryo) plus immune therapy with ipilimumab (ipi) in early stage breast cancer (ESBC). ASCO Breast Symposium; 2014 September 6, 2014; San Francisco, CA.

Petrelli F, Coinu A, Borgonovo K, Cabiddu M, Ghilardi M, Lonati V, Barni S. The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat 2014 144:223-32.

Rapidis AD, Wolf GT. Immunotherapy of head and neck cancer: current and future considerations. J Oncol 2009 9;2009:346-345.

Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G et al. Harmonization of the evaluation of tumor infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs-working group 2014. Ann Oncol. 2014. doi:10.1093/annonc/mdu450.

Schilling B, Harasymczuk M, Schuler P, et al. IRX-2, a novel biologic, favors the expansion of T effector over T regulatory cells in a human tumor microenvironment model. J Mol Med (Berl) 2012;90:139-147.

Schilling B, Harasymczuk M, Schuler P, et al. IRX-2, a novel immunotherapeutic, enhances functions of human dendritic cells. PLoS One 2013;8:e47234.

Słotwińska SM, Słotwiński R. Host response, malnutrition and oral diseases. Part 1. Cent Eur J Immunol. 2014;39(4):518-21

Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst. 2002;94:252-66.

Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568-71.

Whiteside TL, Butterfield LH, Naylor PH, et al. A short course of neoadjuvant IRX-2 induces changes in peripheral blood lymphocyte subsets of patients with head and neck squamous cell carcinoma. Cancer Immunol Immunother 2012;61:783-788.

Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab. 2007;51(4):301-23.

Wolf GT, Fee WE, Dolan RW, et al. Novel neoadjuvant immunotherapy regimen safety and survival in head and neck squamous cell cancer. Head Neck 2011;33:1666-1674.

APPENDICES

Appendix 1: ECOG Performance Status

Score	Performance
100	Normal: no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self; unable to carry on normal activity or do active work.
60	Requires occasional assistance from others; but able to care for most needs.
50	Requires considerable assistance from others, frequent medical care.
40	Disabled: requires special care and assistance.
30	Severely disabled: hospitalization indicated, death not imminent.
20	Very sick, hospitalization necessary, active supportive treatment necessary.
10	Moribund: fatal processes are progressing rapidly.
0	Dead

Source: Karnofsky D, Abelman W, Craver L, Burchenal J. The use of nitrogen mustards in the palliative treatment of carcinoma. Cancer 1948;1:634-56.

Appendix 2: Calculation of Creatinine Clearance by Cockcroft-Gault Equation

Formula: Creatinine Clearance = Sex (Male = 1.0, Female = 0.85) * ((140 - Age) / (Serum Creatinine)) * (Weight / 72)

Online calculator may be found at

http://reference.medscape.com/calculator/creatinine-clearance-cockcroft-gault