USC Norris Comprehensive Cancer Center
Part of the Keck School of Medicine of USC

Local Protocol #: 5GYN-16-2

Protocol Title: A Two-Cohort, Open Label, Phase 2 Trial of the IRX 2 Regimen in Women with Squamous Cervical Intraepithelial Neoplasia 3 (CIN 3) or Vulvar Intraepithelial Neoplasia 3 (VIN 3)
Protocol version date: 08August2020

ClinicalTrialsgov #: NCT03267680

Title:	A Two-Cohort, Open-label, Phase 2 Trial of the IRX-2 Regimen in Women with Squamous Cervical Intraepithelial Neoplasia 3 (CIN 3) or Vulvar Intraepithelial Neoplasia 3 (VIN 3) Protocol: 5GYN-16-2
BB IND #:	133686
Date:	August 08, 2020
Sponsor:	USC Norris Comprehensive Cancer Center Keck Hospital of USC, and LAC+USC Hospital Clinical Investigation Support Office 1441 Eastlake Avenue, Suite 7310 Los Angeles, CA 90033 Phone: (323) 865-0451 Fax: (323) 865-0089 Phone: (212) 582-5352 Email: CoreCISO@med.usc.edu
Principal Investigator:	Lynda Roman, MD Division of Gynecologic Oncology Department of Obstetrics & Gynecology USC Norris Comprehensive Cancer Center, Keck Hospital of USC, and LAC+USC Hospital 1441 Eastlake Avenue, NTT 3411 Los Angeles, CA 90033 Phone: (323) 865-3922
Co-Principal Investigators:	Koji Matsuo, MD, PhD (Clinical) Diane Da Silva, PhD (Laboratory) W. Martin Kast, PhD (Laboratory)
Co-Investigators:	Laila Muderspach, MD Annie Yessaian, MD Huyen Pham, MD Laurie Brunette, MD Susan Groshen, PhD Saloni Walia, MD Marcia Ciccone, MD

Protocol Synopsis

<table>
<tr><td>Study Title</td><td>A Two-Cohort, Open-label, Phase 2 Trial of the IRX-2 Regimen in Women with Squamous Cervical Intraepithelial Neoplasia 3 (CIN 3) or Squamous Vulvar Intraepithelial Neoplasia 3 (VIN 3)</td></tr>
<tr><td>Study Number</td><td>5GYN-16-2</td></tr>
<tr><td>Study Phase</td><td>2</td></tr>
<tr><td>IRX-2 Regimen</td><td>IRX-2 is a primary cell-derived biologic with multiple active cytokine components, produced under pharmaceutical standards from phytohemagglutinin (PHA) and ciprofloxacin stimulated donor mononuclear cells.
In this open label trial, a modified IRX-2 Regimen (as shown in the table below) will include cyclophosphamide on Day 1 (three days before the start of IRX-2), IRX-2 daily x 4 days, and indomethacin, zinc with multivitamins, and a proton pump inhibitor (omeprazole) for 21 days.
All treatments will be repeated at 6 weeks, for a total of two cycles.
<table>
<tr><th>Agent</th><th>Dose</th><th>Route of Administration</th><th>Treatment Days (each cycle) Cycle = 6 weeks</th></tr>
<tr><td>Cyclophosphamide</td><td>300 mg/m^2</td><td>IV</td><td>Day 1</td></tr>
<tr><td>IRX-2</td><td>230 units daily (in 2 ml) in 4 divided doses: 57.5 units (1/2 ml) in each quadrant of the cervix or affected areas of the vulva</td><td>Submucosal injection in the cervix; subcutaneous for vulvar lesions</td><td>4 consecutive days between D4 and D7</td></tr>
<tr><td>Indomethacin</td><td>25 mg TID</td><td>Oral</td><td>Days 1-21</td></tr>
<tr><td>Zinc with multivitamins</td><td>1 tab daily</td><td>Oral</td><td>Days 1-21</td></tr>
<tr><td>Omeprazole</td><td>20 mg daily</td><td>Oral</td><td>Days 1-21</td></tr>
</table></td></tr>
<tr><td>Route of Administration of IRX-2</td><td>For cervical dysplasia: IRX-2 will be administered submucosally in the cervix in four divided doses, one into each quadrant of the cervix, irrespective of location of the CIN 3 lesion.
For vulvar dysplasia: IRX-2 will be administered into the subcutaneous tissue beneath the vulvar lesion. Doses may be divided at clinician discretion based on the geometry and size of the dysplastic lesion.</td></tr>
<tr><td>Reference Therapy</td><td>There will be no reference therapy.</td></tr>
<tr><td>Study Design</td><td>This trial was originally designed as a double-blinded randomized, placebo-controlled study of the IRX-2 Regimen in subjects with CIN 3 or VIN 3. After conversion to an open label, single-arm trial, this study will use a Simon two-stage design for both the CIN 3 Cohort and VIN 3 Cohort.
CIN Cohort: N = 10+12
If 2 or fewer pathological responses are observed among the first 10 patients, the enrollment will be stopped. If 3 or more responses are observed, the trial will continue to a total of 22 CIN patients.</td></tr>
</table>

	• VIN Cohort: N = 5+5 o If there is no pathological response observed among the first 5 patients, the enrollment will be stopped. If 1 or more responses are observed the trial will continue to a total of 10 VIN patients. The primary end-point is pathologic objective response at week 25. CIN subjects will be assessed with colposcopy and photography at baseline. The first six-week cycle treatment will be administered, after which the colposcopy and photography assessment will be repeated, and then a second cycle of treatment will be administered. Colposcopy and photography assessment will be performed again after the second six-week cycle at approximately Week 13 prior to LEEP or wide local excision of CIN lesion at week 25. HPV status will be determined at 4-months post-surgical excision. VIN subjects will be assessed with colposcopy and photography of the vulvar lesion at screening and subsequently on Weeks 6 and 13, followed by wide local excision at week 25.HPV status will be determined at 4-months post-surgical excision. Secondary endpoints include toxicity, feasibility and exploratory immune changes. The protocol Study Team (PI, Co-PI's, Study Monitor, and Study Statistician) will meet monthly and additionally as needed to review the status of subjects and the toxicities. The USC/Norris Comprehensive Cancer Center DSMB will review the progress and safety of the trial every 6 months. All specimens will be analyzed for VIN or CIN (present/absent), grade of dysplasia, HPV virus (present/absent), and nature of the immune infiltrate by immunohistochemistry and gene expression profiling. Peripheral blood will be analyzed by ELISPOT and/or intracellular cytokine analysis for HPV T cell and other cellular responses as well as by flow cytometry and by TCR clonality studies.
Projected Enrollment	22 subjects with CIN 3 and 10 subjects with VIN 3 will be enrolled.
Primary Objective **Primary Endpoint**	To compare the proportion of subjects who achieve a pathologic complete response (CR) and/or a partial response (PR) at weeks 25 based on LEEP or vulvar surgical excision. Each cohort (CIN and VIN) will be assessed independently. The primary endpoint will be occurrence of a pathologic CR or PR of CIN 3 or VIN 3 lesions in the Week 25 excisional specimen. CR is defined as absence of CIN. PR is a lower grade of dysplasia than present at baseline, i.e. grade 3 decreasing to grade 1.

Secondary Objectives	1. To evaluate the toxicity and feasibility of administration of IRX-2 in subjects with confirmed CIN 3 or VIN 3.
	2. To evaluate multiple parameters to assess the clinical and immune activity of the IRX-2 Regimen for the treatment of CIN 3 or VIN 3.
	  a. The occurrence of clinical CRs or PRs at weeks 6, 13 and 25
	  b. Frequency of elimination of HPV in cervical or vulvar tissue using a commercial HPV genotyping assay and viral load determination by quantitative PCR.

	c. Analysis of the immune infiltrates in the resected surgical specimens. Parameters to be evaluated include quantitation of lymphocyte subset infiltration including CD8 and CD4 FOXP3 cells, evaluation of PDL1 expression, evaluation of state of activation and location of infiltrating regions with respect to dysplasia and adjacent normal tissue. A commercial multiplex IHC assay will assess markers of Langerhans cells (CD1a, E-cadherin) and antigen presenting cell (APC) activation (CD80 and CD86). TCR clonality studies may also be performed. d. Immunophenotypic analysis of peripheral blood lymphocytes. Peripheral blood lymphocytes will be evaluated by direct ex-vivo ELISPOT analysis for evidence of induction of γ-Interferon expressing T cells in response to HPV antigens compared to baseline. Changes in naïve, memory, effector and regulatory T cell subset will be analyzed using immunophenotypic flow cytometry. e. Frequency of serum antibodies to HPV E6, E7 and L1 proteins by ELISA. Serum cytokine profile will be analyzed by multiplex ELISA at baseline and week 13 (pending availability of the antibodies). f. RNA expression profiling of immune-inflammatory markers from post-treatment resected surgical specimens. g. Frequency of long term HPV clearance following surgical excision.
Subject Population	Subjects with histologically confirmed squamous CIN 3 or VIN 3.
Participating Center(s)	University of Southern California (Clinical PI, Dr. Lynda Roman; Clinical Co-PI, Dr. Koji Matsuo; Laboratory Co-PIs, Dr. Diane Da Silva and Dr. Martin Kast) University of Oklahoma Health Science Center, Stephenson Cancer Center (PI: Kathleen Moore MD, Co-I's: Joan Walker MD, Katie Smith MD)
Inclusion Criteria:	1. Histologically confirmed squamous CIN 3, or VIN 3 (usual type only). 2. Age ≥25 years. 3. Surgically sterile, postmenopausal, or agrees to practice an effective method of birth control as determined by the investigator (to be continued for one year following last dose of study medication), except that subjects with CIN 3 are not permitted to use a cervical cap or diaphragm for contraception. 4. In good health based upon the results of a medical history, physical examination, vital signs, and laboratory profile including: *Hematology:* • White blood cell >2,500/ mcL (>2.5 x 10^9/L) • Absolute neutrophil count >1,000/ mcL (>1 x 10^9/L) • Platelet count >75,000/ mcL (>75 x 10^9/L) • Hemoglobin ≥8 g/dL (≥80 g/L) (subjects who have received a transfusion or erythropoietin up to one week prior to receiving the first dose of cyclophosphamide are eligible for the study) *Clotting Time:* • International normalized ration (INR) or prothrombin time (PT) ≤1.5 x ULN (upper limit of normal)

	• Activated partial thromboplastin time (aPTT) ≤1.5 x ULN *Renal Function:* • Serum creatinine ≤1.5 x ULN *Hepatic function*: • Total bilirubin ≤2.0 x ULN unless thought to be related to inherited bilirubin conjugation disorder (ie Gilbert's disease) • ALT and AST ≤2.0 x ULN 5. Geographically accessible for ongoing follow-up and committed to comply with the designated visits. 6. Capable of understanding and complying with the protocol and has given written informed consent.
Exclusion Criteria:	1. For subjects with cervical dysplasia: evidence of atypical glandular cells or adenocarcinoma in situ (ACIS) based on cervical cytology, colposcopy or biopsy. 2. For subjects with either cervical or vulvar squamous dysplasia: evidence of microinvasive squamous carcinoma based on cytology, colposcopy or biopsy. 3. For VIN subjects, those with >2 vulvar lesions. 4. Pregnancy or lactation. 5. Allergy to ciprofloxacin or other quinolones (because ciprofloxacin is used in preparation of IRX-2.) 6. Allergy to indomethacin (a component of the IRX-2 Regimen) or to acetylsalicylic acid (aspirin) due to possible cross reaction. 7. Imiquimod for the topical treatment of lower genital tract warts or dysplasia within 3 months of study enrollment. 8. Known positive for human immunodeficiency virus-1 antibody, human immunodeficiency virus-2 antibody, hepatitis B surface antigen, or hepatitis C virus. 9. Known to have other immunodeficiency diseases, including cellular immunodeficiencies, hypogammaglobulinemia, or dysgammaglobulinemia. 10. Immunotherapy (eg, interferons, tumor necrosis factor, interleukins, or biological response modifiers [granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor) or any investigational drug within 3 months of study enrollment. 11. Concurrent treatment with systemic corticosteroids at a dose of ≥5 mg/day of prednisone (or equivalent). An infectious process or any other significant illness such as an autoimmune disease, comorbidities or advanced age that in the opinion of the investigator would compromise the subject's ability to mount an immune response. 12. Subjects should not take aspirin (except for low-dose aspirin as prescribed for vascular disease) or other non-prescribed, non-steroidal anti-inflammatory agents from start of treatment to surgery. 13. An infectious process or any other significant illness such as an autoimmune disease or advanced age that in the opinion of the investigator would compromise the subject's ability to mount an immune response. 14. Active SARS-CoV2 infection or COVID-19 disease 15. Impaired hepatic, renal or hematological function, evidenced by: a. Alanine aminotransferase, aspartate aminotransferase, or total bilirubin (in the absence of Gilbert's syndrome) >2 times upper limit of normal, b. Serum creatinine >1.5 times upper limit of normal, or

	16. Clinically significant active cardiovascular disease, including a history of myocardial infarction within the past 6 months, heart failure as defined by New York Heart Association classes III or IV, and/or blood pressure greater than 160/100 mm. 17. History of severe allergic reaction to insect bites or stings, or to any biologic pharmaceutical product, including compounds similar to the test article. 18. Any medical contraindications, allergies or previous therapy that would preclude treatment with the components of the IRX-2 Regimen, i.e., cyclophosphamide, indomethacin, zinc-containing multivitamins or omeprazole. 19. Donation or loss of >450 mL of blood or plasma within 30 days of start of treatment
Immunologic and Virologic Assessments	Presence or absence of cervical HPV (using cervical swabs) for all subjects will be determined at Screening/Baseline, at Week 25, and 4 months post excision (Week 41). HPV genotype will be determined by analyzing the Screening/Baseline biopsy and the Week 25 surgical excision. Immunophenotypic analysis of peripheral blood lymphocytes will be performed at Screening/Baseline; at Week 6, 13 and 25 by ELISPOT analyses using HPV peptide pools and assessment of cytokine release from HPV–specific T cells Enzyme-linked immunosorbent measurements of serum to determine the HPV antibody response will be performed at Screening/Baseline; Week 6, 13 and 25 pending the availability of appropriate reagents. The Screening/Baseline biopsy and the subsequent cervical LEEP or vulvar excisional specimen will be compared to evaluate alterations in the tissue immune environment including T cell effector and T-regulatory and APC subsets looking at differentiation markers and markers of activation will be assessed dependent on obtaining adequate size tissue. Multiplex flow cytometry and nanostring gene expression profiling will be performed.
Statistical Considerations	The CIN and VIN cohorts will be analyzed separately. Within each cohort, all patients who received any amount of treatment will be included in the summaries for primary and secondary endpoints with standard descriptive methods. For CIN Cohort, with a false-positive error rate of 5% when the true objective response rate is 20% and a false-negative error rate of 10% when the true CR + PR response rate is 50%, a total of 10 patients will be enrolled in the 1st stage. If 2 or fewer responses are recorded among the first 10 patients to receive the IRX Regimen, the enrollment will be stopped. If 3 or more responses are observed, the trial will continue to a total of 22 CIN patients. For the VIN Cohort, with a false-positive error rate of 10% when the true objective response rate is 5% and a false-negative error rate of 10% when the true CR + PR response rate is 40%, a total of 5 patients will be enrolled in the 1st stage. If there is no response observed among the first 5 patients, the enrollment will be stopped. If 1 or more responses are observed the trial will continue to a total of 10 VIN patients. The primary endpoint is the response, CR or PR of CIN 3 or VIN 3 lesions to the IRX-2 Regimen as determined by histologic examination of the Week 25 surgical excision specimen. Safety assessments will be summarized using descriptive statistics for continuous measures and frequency distributions for categorical responses. Differences between groups will be compared using standard statistical tests for small samples.

	Immune assessment of peripheral blood lymphocytes will include determination of the percentage of γ-IFN secreting T cells by ELISPOT in response to various HPV epitopes. Responses will be considered positive if there is a 2x increase in the number of γ-IFN secreting cells over Baseline. Differences between groups will be compared using standard student's T test. Immune infiltrates in cervical and vulvar biopsy specimens will be summarized and differences between groups will also be compared using standard student's T test.

Table 1: Schedule of Events

Study Procedure	Screening (Baseline)	Treatment Cycles																				Pre-op procedure visit	Surgical Resection (LEEP or wide local excision)	Post-Op Check[i]	4-month post-op
		Cycle 1: 1st 6-weeks									Cycle 2: 2nd 6-weeks									Week 13 safety visit				
	Days −30 to 0	Day(s) of Cycle 1									Days(s) of Cycle 2										Week 21	Week 25	Weeks 26+	Week 41
		1	2	3	4	5	6	7	>8		1	2	3	4	5	6	7	>8							
Day(s) of Protocol		1	2	3	4	5	6	7	8-21	22-42	43	44	45	46	47	48	49	50-63	64-84	91	Day 147 ± 14 days	Day 175 ± 7 days[g]		Day 290 ± 14 days
Informed consent	X																							
Study Drug Administration																								
Cyclophosphamide IV[a]		X									X													
Indomethacin, omeprazole, zinc with multivitamins[b]		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X						
IRX-2 injection[b]					X	X	X	X						X	X	X	X							
Procedures & Evaluations																								
History & Complete Physical Exam	X										X									X	X			
HIV-1, HIV-2, HBV, HCV	X																							
SARS-CoV2		X[c]									X[c]													
Focused Pelvic exam																						X	X[j]	X
Injection Site Check					X			X						X			X			X	X			
ECOG Performance Status	X	X						X			X						X			X	X		X	X
AEs and SAEs[d]		X			X	X	X	X			X			X	X	X	X			X	X		X	X
Concomitant Medications	X	X			X	X	X	X			X			X	X	X	X			X	X		X	X
Chemistry, CBC, PT/INR	X	X[e]									X[e]									X[e]	X[e]			
Blood work for investigational studies[f]	X[e]	X[e]									X[e]									X[e]	X[e]			
Blood for PK studies[h]					X	X	X	X						X	X	X	X							
Pregnancy Test[f]	X	X[f]									X[f]									X[f]	X[f]			

Colposcopy & cervix photography (CIN patients)	X										X									X	X			
Cervical inspection & photography (CIN patients)					X[L]			X[L]						X[L]			X[L]							
Cervical cytology (pap smear/ECC) (CIN patients)																				X	X			X
Vulvar inspection & photography (VIN patients)	X				X[L]			X[L]			X			X[L]			X[L]			X	X			
Cervical or vulvar swab for HPV assay (research)	X																			X	X			X
Cervical or vulvar biopsy	X[k]																							
Cervical LEEP excision or cone biopsy or vulvar excision or biopsy																						X		

Footnotes:

a. Dexamethasone or other steroids should not be used as an anti-emetic with the cyclophosphamide. Steroid administration may inhibit IRX-2 Regimen stimulation of an immune response. The prescribed dose is significantly less than used for conventional chemotherapy and thus steroids will not be needed. Suggested antiemetic regimen would include a serotonin (5-HT3) antagonist and lorazepam.
b. Subjects will receive injection of study agent IRX-2 daily on Days 4, 5, 6, 7 or Days 5, 6, 7, 8. The indomethacin, omeprazole, zinc with multivitamins are administered for the first 3 weeks per cycle, on Days 1-21. For cycle 2 [Week 7] the comparable "Days" are shown in the table.
c. SARS-CoV2 virus test required within 2-4 days prior to start of Cycle 1 and Cycle 2. If positive, treatment will be held.
d. Adverse events (AEs) and serious adverse events (SAEs) are measured from day of Cycle 1 Day 1 until Week 25 of the study. Ongoing AE/SAEs will be reported and monitored until resolution.
e. Ten ACD yellow top blood tubes (85 mL) and one red top serum tube should be drawn for investigational studies. Blood work can be done up to seven days prior to the specified day. Blood draws do not need to be repeated on indicated day if drawn within the previous 7 days.
f. Pregnancy must be ruled out, or post-menopausal status verified, at study enrollment. Fertile subjects will need effective contraception during the study and for 1 year following last dose of study medication; investigator to repeat pregnancy tests during the study if needed for verification. A blood pregnancy test should be used at screening, week 7 and week 21. A urine pregnancy test can be performed on Cycle 1 day 1 visit, and at week 13 safety visit.
g. The "Week 25" lesion excision is to be scheduled for Day 175, +/- 7 days.
h. One red top serum tube (5 mL) required at each of the following time points: Day 4 pre-injection, and then 30 minutes, 1 hour, 2 hours post-injection; Day 5 pre-injection; Day 6 pre-injection; Day 7 pre-injection, and then 30 minutes, 1 hour, 2 hours post-injection. All time points allow +/- 5 minutes.
i. Post-op check to be performed 1 to 8 weeks post-surgery; can occur by phone or office visit.
j. If seen for office visit.
k. If previous biopsy not done within past 60 days, or if slides/block from this are not available, or at discretion of investigator based on clinical impression.
l. Photography to be performed prior to injection of study drug.

Table 2: Schedule of Virologic and Immune Monitoring (All Subjects) Based on Blood, Swab or Tissue Samples

Study Procedure	Screening (Baseline)	Treatment Cycles		Safety visit	Surgical Excision	4-month follow-up
		Cycle 1	Cycle 2	Week 13	Week 25	Week 41
	Days -30 to 0	Day 1	Day 43 (Cycle 2, Day 1)	Day 91	Day 175 ± 7 days	Day 290 ± 14 days
Presence or Absence of HPV[c]	X				X	X
HPV Genotyping[c]	X				X	X
Flow Cytometry[c]	X	X[a,b]	X[b]	X[b]	X[b]	
ELISPOT[c]	X	X[a,b]	X[b]	X[b]	X[b]	
ELISA[c]	X	X[a,b]	X[b]	X[b]	X[b]	
Phenotypic analysis of lymphocytic infiltrate[d]	X				X	

a. Cycle 1, Day 1 labs do not need to be repeated if the Screening (Baseline) labs were drawn within seven days. For subject convenience, investigator should synchronize these phlebotomies with the blood draws specified in Table 1.
b. Blood work for ELISPOT, ELISA and flow cytometry can be collected up to seven days prior to the specified day.
c. HPV genotyping, flow cytometry, ELISPOT, and ELISA assays will be performed in the Norris Comprehensive Cancer Center Immune Monitoring Core laboratory. Immunogenicity assays will be performed by Brooklyn ImmunoTherapeutics on stored serum.
d. Tissue immunohistochemistry and PK studies will be done by Brooklyn ImmunoTherapeutics via a third party.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

List of Definitions and Abbreviations

ACIS	adenocarcinoma *in situ* (of the cervix, in the context of this protocol)
ACOG	American College of Obstetricians and Gynecologists
AE	adverse event
Akt	protein kinase B
ALT/SGPT	alanine aminotransferase/serum glutamic pyruvic transaminase
ASCCP	American Society for Colposcopy and Cervical Pathology
AST/SGOT	aspartate aminotransferase/ serum glutamic oxaloacetic transaminase
BUN	blood urea nitrogen
CAP	College of American Pathology
CBC	complete blood count
CD	cluster of differentiation
CFR	Code of Federal Regulations
CIN 3	cervical intraepithelial neoplasia 3; comparable nomenclature is severe cervical dysplasia and cervical carcinoma *in situ*.
CIS	carcinoma *in situ*; see also CIN 3, VIN 3. Usually refers to squamous cell histology, in contrast to adenocarcinoma *in situ* (ACIS)
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
CRO	Contract Research Organization
DSMB	Data and Safety Monitoring Board
ECC	endocervical curettage
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
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 for CIN or VIN	Sublesional injections of 230 U of IRX-2 for 4 days, preceded by cyclophosphamide with a 21-day course of indomethacin and zinc containing multivitamins; repeat cycle once in 6 weeks
IV	intravenous
IxRS	Interactive Recognition System
LAST	Lower Anogenital Squamous Terminology
LC	Langerhans cell(s)
LDH	lactate dehydrogenase
LEEP	large loop excision procedure (for excision of cervical dysplasia)
LLETZ	large loop excision of the transformation zone
LSIL	low-grade squamous intraepithelial lesion
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK or NKT	natural killer T cells
PI	Principal Investigator
PI3-K	phosphoinositide 3 kinase
PK	pharmacokinetic
PS	performance status
PT	prothrombin time
PTT	partial thromboplastin time
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIL	squamous intraepithelial lesion
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 c ell
TH	T-helper
TH_1	T-helper 1 (cell mediated)
TH_2	T-helper 2 (humoral)
TID	three times a day
TNF-α	tumor necrosis factor alpha
U	unit(s)
ULN	upper limit of normal
US	United States
VIN 3	Vulvar intraepithelial neoplasia 3; comparable nomenclature is severe vulvar dysplasia and vulvar carcinoma *in situ*.
WLE	wide local excision

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Female Lower Genital Tract Dysplasia

Lower genital tract dysplasia in women encompasses premalignant conditions of the uterine cervix, vagina, vulva and adjacent perineal skin. Dysplasia of perianal tissues and anus are now recognized as part of the spectrum of this disorder due to common anatomic origin from the embryonic cloaca and shared etiology of antecedent infection with an oncogenic strain of human papilloma virus (HPV) [Schiffman, 1993; Darragh, 2013]. Lower anogenital dysplasia and lower genital tract dysplasia are used interchangeably in this introductory section.

Untreated lower genital tract dysplastic lesions can progress to invasive cancers. Almost all cervical and vaginal cancers develop from pre-existing dysplasia, as do the majority of vulvar and anal cancers. Current Pap smear screening programs (for cervical dysplasia) and better recognition of visible or symptomatic lesions (for vulvar dysplasia) have improved the diagnosis of these premalignant lesions, at least in developed countries with the resources needed for this clinical care. Clinicians also now understand that infection with an oncogenic type of HPV is almost always the first step towards malignant transformation of anogenital epithelium. Unfortunately, HPV is endemic among sexually experienced individuals. While the risk does correlate with number of lifetime partners, it remains relatively high (4 to 20 %) even in those with one partner. Overall, at least 75 to 80% of sexually active women will have acquired a genital HPV infection by age 50 [Workowski, 2015]. Vaccines directed against HPV 16 and HPV 18 (2 of the more oncogenic HPV types) were introduced over 10 years ago, and a vaccine that also covers HPV types 31, 33, 45, 52 and 58 is now available [FDA, 2014]. Vaccination against these oncogenic types of HPV could prevent up to 90% of cervical cancers, with comparable reduction in the incidence of dysplasia and invasive cancer of other lower genital tract sites. However, current HPV vaccination programs will only benefit the generation of young woman eligible for vaccination. And to date the vaccination rate in the USA is only about 30%, which is below the level needed for protective herd immunity [Widgren, 2011; CDC, 2013]. Thus there remains an ongoing need for effective treatment to prevent the morbidity and mortality of HPV-associated lower genital tract dysplasia and cancer.

Despite substantial gains in understanding the molecular biology of HPV-associated carcinogenesis [Schiffman, 1993; Alani, 1998; Darragh, 2013], the treatment of lower genital tract dysplasia has not improved. Clinicians use variations of the same crude ablative or excisional techniques dating to the 1950's to eradicate dysplastic lesions [Montz, 2000; Martin-Hirsch, 2013; ACOG, 2011]. As detailed below, existing treatments of cervical and vulvar dysplasia cause substantial short term morbidity as well as long term risks to fertility, pregnancy outcomes, and sexual function. Furthermore, failure rates of these procedures are high, in part due to the multifocal nature of lower anogenital tract dysplasia; thus even women with apparent eradication of a dysplastic lesion remain at risk for recurrent dysplasia and cancer decades into the future [ACOG, 2012]. This is a particular risk for women with a history of VIN 3, of whom one-third will experience a recurrence of dysplasia, and 4 to 8% will eventually develop invasive cancer [van Seters, 2005].

The limitations of existing treatments for cervical and vulvar dysplasia led us to develop this Phase 2 research protocol to evaluate the novel immunotherapy regimen, IRX-2, for this patient

population. Women with severe cervical or vulvar dysplasia (CIN 3, VIN 3) will be invited to participate. All subjects will undergo the medically indicated surgical excision of their dysplastic lesion; subjects in the experimental arm will also receive sublesional injections of IRX-2. While this initial exploratory trial will of necessity have limited objectives, our hope is that our research can eventually achieve three goals:

- Avoid large and potentially dangerous or disfiguring excisions currently required for conventional treatment of lower genital tract dysplasia.
- Restore the subject's immune system to prevent recurrent dysplasia and decrease future risk of secondary lower genital tract cancers. Multiple lines of experimental and clinical evidence have demonstrated the influence of the immune system upon relapse rates for lower genital tract dysplasia [Freiser, 2013; Gildener-Leapman, 2013]. Thus enhancing immunologic control of the pre-invasive lesions can confer benefits well beyond the initial excision of the dysplastic lesion.
- Finally, if this IRX-2 Regimen is proven to be effective for eradicating HPV-related dysplasia, then it may also have a role in the adjuvant treatment of anogenital tract cancers.

In the following sections, the diagnosis and management of cervical and vulvar dysplasia will be further reviewed, followed by a discussion of the interplay between HPV infection and local cellular immune-deficiencies.

The IRX-2 Regimen has also been studied in a pilot study of 10 subjects with early stage cervical cancer as neoadjuvant, pre-operative therapy [Dueñas-Gonzalez, 2002]. This trial provides important data demonstrating the safety and feasibility of the IRX Regimen treatment of cervical disease. The treatment was well tolerated except for mild pain and minor bleeding during injections and gastric intolerance to indomethacin. Clinical response was seen in 50% of subjects (three partial responses, two minor responses). In addition, IRX-2 had been shown in vitro to induce significant upregulation of the immune response directed against human papilloma virus (HPV)-exposed Langerhans cells, thereby potentially preventing HPV-induced cancers [Da Silva, 2016].

1.1.2 Nomenclature: Dysplasia, Intraepithelial Neoplasia, and Squamous Intraepithelial Lesion

Historically, premalignant squamous lesions of the uterine cervix were described as mild, moderate or severe cervical dysplasia. Carcinoma-in-situ was used by most pathologists to denote full thickness replacement of the epithelium with severe dysplasia. Lesions of the vagina, vulva, perineum, and anus were similarly categorized. Several terminology systems have been introduced over the decades to allow better inter-observer diagnostic accuracy and more uniform reporting, clarify the distinction between cytology reports for Pap smears and histology reports for biopsies, and address the difficulties associated with the diagnosis of glandular neoplasia of the cervix [Muntz, 2004].

1.1.2.1 Bethesda System for Grading Cervical Dysplasia

For cervical dysplasia, the Bethesda system is most commonly used; its current iteration was released in 2001 [Solomon, 2002]. Pap smear cytology is described using the term "squamous intraepithelial lesion" (SIL) and histologic changes are described using the term "cervical intraepithelial neoplasia" (CIN). There are three degrees of severity for CIN:

1. CIN 1 is a low grade lesion. A biopsy reveals mildly atypical cellular changes in the lower third of the epithelium. Koilocytotic atypia due to the cytopathic effect of HPV infection is usually present.
2. CIN 2 is a higher grade lesion. A biopsy reveals moderately atypical cellular changes confined to the lower two-thirds of the epithelium with preservation of epithelial maturation. CIN 2 is analogous to moderate dysplasia. There is considerable diagnostic variability in this category. Clinical guidelines [Massad, 2013] used for treatment assume the cancer risk posed by CIN 2, while lower than the risk for CIN 3, is high enough to warrant treatment; nonetheless, many CIN 2 lesions have a low risk of malignant progression and will regress without treatment.
3. CIN 3 is a high grade lesion with a substantial risk of progression to cancer if untreated. Histology reveals severely atypical cellular changes encompassing more than two-thirds of the epithelial thickness and up to full-thickness involvement. CIN 3 is analogous to severe dysplasia or carcinoma in situ.

1.1.2.2 Glandular Dysplasia, Adenocarcinoma-in-situ, and Adenocarcinoma of the Uterine Cervix

The ectocervix, or the portion of the cervix that is visualized with a vaginal speculum examination, is covered by squamous epithelium. The cervical canal (endocervix) is covered with glandular epithelium. While dysplasia of both squamous and glandular tissues share the same HPV-mediated pathogenesis, recognizing and categorizing the severity of glandular dysplasia is very difficult. For that reason the Bethesda system specifically excludes glandular dysplasia from the CIN nomenclature; e.g. CIN refers only to squamous abnormalities [Solomon, 2002].

The Bethesda system does provide guidance for reporting abnormal glandular cells detected by screening cytology Pap smears as well as biopsies showing glandular abnormalities. The preferred nomenclature is “atypical glandular cells” (AGC) for reporting abnormal glandular cytology, with “AGC favor neoplasia” used for more worrisome findings. But AGC interpretations are poorly reproducible, even by expert cytopathologists [Lee, 2002]. The underlying histologic abnormality may often be a squamous lesion. The glandular lesions associated with AGC cytology can range from benign polyps to low grade abnormalities to adenocarcinoma-in-situ to invasive adenocarcinoma of the uterine cervix; furthermore, an AGC pap smear can be caused by occult adenocarcinomas of the endometrium, fallopian tube, ovary, and other sites [Zhao, 2009]. Careful diagnostic evaluation is required for any patient with an AGC pap smear report, including extensive biopsies since glandular dysplasia is difficult to detect by colposcopy. There is also a small but important risk of occult adenocarcinoma; for example, in a large population data base, AGC pap smears in women over the age of 30 were due to occult invasive adenocarcinoma in 3% of cases [Katki, 2013]. Therefore it is prudent to excise a dysplastic glandular lesion without undue delay. Furthermore, if histology establishes adenocarcinoma-in-situ as the correct diagnosis, then hysterectomy remains the treatment of choice for women who have completed childbearing [Muntz, 1992; Muntz, 1996; Massad, 2013].

For all of these reasons, women with severe glandular dysplasia or adenocarcinoma in situ of the uterine cervix – even though this is the glandular corollary to squamous CIN 3 – are NOT eligible for participation in this clinical trial.

1.1.2.3 Vulvar Dysplasia

In parallel with the descriptions used for cervical dysplasia, vulvar dysplasia has been historically divided into three grades: mild, moderate and severe (with the latter including carcinoma in situ). Since the late 1980's the Bethesda nomenclature of intraepithelial neoplasia has been preferred, e.g. VIN 1, VIN 2 and VIN 3. As with CIN, all VIN lesions are by definition derived from squamous tissue, and are the most common precursors for vulvar squamous cell carcinoma. Unlike cervical cancers, of which a substantial proportion is adenocarcinoma, over 90% of vulvar cancers are primary squamous cell carcinomas. The few non-squamous categories include rare Bartholin gland cancers, adenocarcinomas arising from vulvar Paget's disease, primary vulvar melanoma, or secondary malignancies from other sites [Fox, 2003].

In 2004 the International Society for the Study of Vulvar Diseases (ISVVD) made important updates to the VIN nomenclature [Sideri, 2005], including the following:

1. There is now substantial evidence that what had been termed VIN 1 is not a cancer precursor requiring treatment [ACOG, 2011]. Thus, VIN 1 was dropped from the nomenclature, and those lesions are referred to as condyloma acuminatum. The term VIN is now limited to high grade squamous lesions (VIN 2 and VIN 3).
2. VIN was classified into two main categories:
    a. VIN, usual type: These are the lesions associated with HPV infection, and are the most common in the general population.
    b. VIN, differentiated type: These lesions comprise less than 5% of VIN and typically occur in postmenopausal women. Differentiated VIN lesions are not associated with HPV infection. The lesions arise from areas of chronic vulvar irritation, most commonly due to vulvar dermatoses such as lichen sclerosus. The clinical, molecular, and immunologic characteristics of the differentiated type of VIN are distinct from those of the usual HPV-mediated type of VIN. For this reason, subjects with the differentiated type of VIN are excluded from this research study.

1.1.2.4 Lower Anogenital Squamous Terminology (LAST) Project

In 2012 the Lower Anogenital Squamous Terminology (LAST) project of the College of American Pathology (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) proposed uniform changes in terminology to describe all HPV-associated squamous lesions of the anogenital tract [Darragh, 2013]. The LAST project dropped the CIN and VIN nomenclature favored by the Bethesda and ISSVD systems for reporting cervical and vulvar histology (as well as VaIN, PAIN and AIN for vaginal, perianal and anal histology, respectively). Instead, the SIL nomenclature is used to report both cytologic and histologic abnormalities. Moderate grades of dysplasia are stratified according to p16 immunostaining to better identify precancerous lesions. Specimens that are p16-negative are categorized as LSIL and those that are p16-positive are categorized as HSIL. For vulvar dysplasia, VIN 2 and VIN 3 are combined into one category: vulvar HSIL.

While there are cogent reasons for the changes proposed by the LAST project, at this time the new terminology is not widely used. Furthermore, clinicians principally rely on the Bethesda system's CIN terminology when using ASCCP guidelines for the evaluation and management of abnormal

cervical cytology and histology [Massad, 2013]. For these reasons the CIN terminology will be used for this research protocol.

Appendix 1 and Appendix 2 provide a comparison between the different nomenclatures used to describe cervical and vulvar dysplasia.

1.1.3 The Management of CIN 3, and its Long Term Sequelae

1.1.3.1 Management of CIN 3

Because of the risk CIN 3 lesions can progress to cancer, treatment requires complete destruction or excision of the dysplastic lesion with adequate margins. Lesion destruction can be done with cryotherapy or CO2 laser vaporization. But in general an excisional procedure is now favored because it is usually better tolerated by the patient and yields a histology specimen for determination of margin status and to rule out the presence of occult invasive carcinoma [Massad, 2013].

Cervical excisions are accomplished with a superficial large loop excision of the transformation zone (LLETZ), also known as a loop electrosurgical excision procedure (LEEP). A deeper electrosurgical excision can also be performed that includes more of the endocervical canal and is commonly referred to as a LEEP conization procedure. These procedures are normally performed in a clinic or office setting; some patients, for instance who cannot tolerate an office procedure, are best managed in an operating room under sedation or anesthesia. Some patients have lesions that require a scalpel cervical conization for an adequate excision; these procedures are performed in an operating room with anesthesia [Montz, 2000; Kyrgiou, 2006; Martin-Hirsch, 2013]

Success rates for excisional procedures for the management of CIN 3 are 90-95% with most failures diagnosed within two years [Paraskevaidis, 2004]. Long-term follow-up is also needed due to the risk that the patient will develop new dysplastic lesions elsewhere within the lower genital tract [Jakobsson, 2009]. With respect to future risk of cervical cancer, women with a history of CIN have a 10-fold increased risk during the next 20-25 years when compared to the general population [Melnikow, 2009; Rebolj, 2012].

A small proportion of patients diagnosed with CIN 3 will have no histologic evidence of dysplasia in the subsequent excisional specimen performed for treatment. Whether this represents complete removal of a small lesion with the initial diagnostic biopsy, the effect of post-biopsy inflammatory changes, or spontaneous resolution of the dysplastic lesion is impossible to ascertain for any one patient. Based on observational studies and clinical trials, estimates of this rate range widely, from less than 5% up to 40%. The variability is best explained by several factors, including:

- The population studied.
    - Women under age 25 have a greater likelihood of clearing a dysplastic lesion without treatment [Moscicki, 2001; Moscicki, 2010; Bosch, 2006];
    - Smokers have a greater risk of persistent disease [Deacon, 2000].
- Inclusion of subjects with varying degrees of dysplasia, for instance CIN 2, 3 vs. CIN 3 only. CIN 2 lesions diagnosed by conventional histology have a higher rate of regression because many are best categorized as low grade SIL. As discussed above, the LAST classification system addresses this by requiring stratification of CIN 2 lesions with p16 staining [Darragh, 2013].

- Different definitions for resolution, such as complete absence of dysplasia in the surgical specimen vs. decrease in the extent or severity of the dysplasia.

For these reasons this study is limited to women age 25 years or older, smoking history is a variable, and both complete and partial responses will be measured. Finally, the statistical model for our study used a modest spontaneous regression rate of 20% when calculating sample size to ensure an adequate number of subjects would be recruited. The spontaneous regression rate will not be as much of a confounding factor in VIN3 disease given the rate is much lower.

1.1.3.2 Complications and Long-Term Sequelae from the Surgical Management of CIN 3

The discomfort and psychological distress caused by the surgical treatment of CIN 3 should not be minimized [Lerner, 2010]. Nonetheless, immediate adverse events such as pain, hemorrhage and infection are uncommon, usually of mild to moderate severity, and manageable with routine clinical care [Martin-Hirsch, 2013]. Of greater concern is that CIN 3 most often affects reproductive-age women and damage to their cervix may impact normal menstruation, fertility and future pregnancy outcomes.

Any surgical procedure can lead to scarring; for cervical excisions, this can result in cervical stenosis. This complication has been reported in up to 8% of women undergoing excisional treatment for CIN [Baldauf, 1996]. Cervical stenosis is linked to a number of complications, including:

- Obstruction of menstrual flow, leading to painful menstruation (dysmenorrhea). In severe cases blood and debris accumulate inside the uterine cavity (hematometra), which can become secondarily infected (pyometra). Surgical management is required (ranging from cervical dilation to hysterectomy).
- Severe cervical stenosis can prevent cervical dilation during labor. Ordinarily this is managed with a cesarean section. In other cases labor proceeds but only after disrupting the scar tissue with deep cervical lacerations followed by massive postpartum hemorrhage.

Removal of cervical tissue from around the cervical os and into the endocervical canal can cause two other effects: structural weakness of the cervix and destruction of the glands that form the protective endocervical mucous plug. Complications linked to these alterations in normal cervical anatomy are summarized below:

- The risk of second-trimester pregnancy loss is significantly increased, presumably due to loss of structural integrity of the cervix following an excisional procedure. This devastating complication usually occurs between 16 and 24 weeks gestational age – long after the mother has bonded with her infant but a few weeks too early for any hope of delivering a healthy live neonate. The baseline population risk of second trimester pregnancy loss is only 0.4%. Based on population studies [Albrechtsen, 2008] and a large meta-analysis [Kyrgiou, 2014] this risk increases significantly to 1.5-1.6% (RR approximately 2.60, 95% CI 1.45-4.67).
- The risk of preterm premature rupture of membranes (PPROM) is significantly increased in women who have undergone a LEEP, rising from 2% to 5% [Kyrgiou, 2006]. This increased risk has been attributed to a short, scarred and inelastic cervix transmitting shearing forces to the fragile amniotic sac.

- There is an increased risk for ascending bacterial invasion of the amniotic sac, leading to preterm delivery and both neonatal and maternal sepsis [Svare, 1992].
- Preterm delivery and perinatal mortality are both increased in most long-term studies evaluating the effects of CIN treatments on pregnancy outcomes. Preterm delivery and perinatal mortality are closely linked, with most perinatal deaths in these studies due to complications of prematurity. The relative risk of preterm delivery seems to vary widely based on the type of excision performed, with perinatal mortality also tracking with gestational age at delivery. Overall, the best risk estimates are derived from a large 20-study meta-analysis [Arbyn, 2008] and a large Danish population-based study [Noehr, 2009]. For example:
    - The meta-analysis linked cold knife conization to an increase in perinatal mortality, which rose from 0.5% to 4.3% (RR 2.9, 95% CI 1.4-5.8]. The increased perinatal mortality rate paralleled an increase in preterm deliveries between 28-34 weeks.
    - The Danish population-based study found an association between LEEP and preterm deliveries throughout the third trimester. This included an increase in deliveries at 32 to 36 weeks (baseline rate 2.9%, increasing to 5.0%). Inclusion of these "late preterm infants" in the Danish study is important. Even though these neonates rarely die (and thus are not reported in mortality statistics), their increased morbidity is still substantially greater than full term infants.

After reviewing these potentially catastrophic complications, is it imperative to remember that the main priority when managing a woman with CIN is to eliminate her risk of dying from cervical cancer. Yet a prudent clinician can mitigate the long term reproductive risks with common sense and by following current guidelines [Massad, 2013], such as:

- Adolescents and young women (< age 25) have a very low risk of cancer. Furthermore, transient HPV infection is common (which even for "high risk" types is not linked to future cancer risk) and dysplastic lesions, if they develop, often regress [Moscicki, 2001; Moscicki, 2010]. For these reasons close observation can be chosen over either ablation or excision for carefully selected patients.
- Ablation techniques (rather than excision) can be used for other young women – loosely defined based on number of children already delivered and future childbearing plans. This is most appropriate for women with more moderate degrees of dysplasia and other favorable risk factors in whom the likelihood of occult invasive cancer is negligible.
- And for women with CIN 3, who will usually need an excisional procedure, the gynecologic surgeon should limit the depth of the excision, and if possible avoid multiple excisions.

Another way to avoid long-term complication is to develop a non-surgical treatment for CIN. If IRX-2 can successfully eradicate CIN 3 then it could become a new treatment option for CIN. This could substantially benefit not only the many women facing a CIN diagnosis but also improve future pregnancy outcomes and neonatal health.

1.1.4 Management of VIN

1.1.4.1 Surgical Excision

Surgical management of VIN 3, similar to the management of CIN 3, requires either destruction (laser vaporization) or excision of the dysplastic lesion; and as with CIN 3, excisional procedures are generally preferred because those allow histologic assessment. Unlike CIN, spontaneous regression of vulvar dysplasia is very uncommon (under 1.5%) and treatment is required for almost all women [van Seters, 2005], excepting only the occasional very young patient with an ambiguous diagnosis favoring benign condyloma accuminata. Furthermore, VIN is often multifocal. The interlabial folds, clitoris, posterior fourchette, perineum and perianal tissues are frequently affected by widely dispersed lesions. More extensive disease can have confluent involvement of the labia majora, labia minora, perineal skin and anus. Multifocal or confluent high grade lesions are present in up to two-thirds of women with VIN, and these are often admixed with lower grade dysplastic lesions and condylomata [Friedrich, 1980].

Each patient therefore will have a unique distribution of abnormal tissue spread over a potentially wide area, and eradicating the dysplastic tissue while preserving normal vulvar anatomy and function can be very difficult [ACOG, 2011]. Small lesions can be managed with a wide local excision followed by reapproximation of the resulting surgical defect. Removing the epidermis with a small amount of underlying dermis is sufficient. Larger lesions require a partial vulvectomy. Extensive multifocal or confluent lesions are managed with a skinning vulvectomy. This procedure is done by removing all of the vulvar skin along the relatively avascular plane between the epidermis and dermis with the large defect covered by a split thickness skin graft [Rutledge, 1968]. Concurrent lower grade lesions and condylomata can be managed with ablative therapy to decrease the amount of vulvar tissue surgically excised. CO2 laser vaporization, argon beam coagulation, and cavitational ultrasonic surgical aspiration (CUSA) can be used for this purpose, although laser vaporization is currently preferred by most gynecologic oncologists [ACOG, 2011].

1.1.4.2 Efficacy of Current VIN Treatments

Regardless of whether excision or ablation (or both) are used, many women require several painful and potentially disfiguring treatments for initial eradication of VIN 3. Even then, approximately 1/3 will develop recurrent VIN [van Seters, 2005]. Risk factors for recurrence include immunosuppression, cigarette smoking, multifocal or multicentric disease, larger lesion size and involved margins. Long-term follow-up is required for decades and must include surveillance of the entire anogenital tract for appearance of new dysplastic lesions. Finally, approximately 3-8% of patients develop invasive squamous cell cancer following treatment for VIN 3 [van Seters, 2005]. So while the overall population risk for vulvar cancer is lower than the risk of cervical cancer (approx. 5,000 vs. 13,000 cases/year in the US in 2015 [Siegel, 2015]), an individual woman's risk of developing invasive cancer after treatment for VIN 3 is significant.

Thus a more effective and less disfiguring treatment with better long term control of dysplasia and prevention of cancer is clearly needed for women diagnosed with VIN 3.

1.1.4.3 Topical Therapy: Imiquimod Cream (Aldara®)

Topical therapies have been tried in an attempt to preserve vulvar anatomy, especially in younger women. Imiquimod cream (Aldara®) is approved for the treatment of vulvar and perianal condyloma accuminata [Aldara Prescribing Information, 2010]. Imiquimod is a topical immune response modifier with antiviral and antitumor effects mediated through the stimulation of local

cytokine production and cell-mediated immunity. A course of imiquimod treatment lasts 16 weeks, during which time the cream is topically applied to the wart. All patients experience blistering and desquamation of vulvar skin to some degree; most are severe enough to require dose modifications and interruption of treatment. "Off-label use" for VIN should be considered investigational but has been somewhat successful, with up to 50% complete response rate reported in a collection of patients (n = 162) pooled from ten small studies [Mahto, 2010]. Indeed the suggestion that the weak immunostimulatory properties of imiquimod can be effective supports our investigation of the cytokine-based IRX-2 Regimen for the treatment of VIN 3.

1.1.4.4 Investigational Therapeutic Vaccination for VIN 3

Preliminary investigations of therapeutic HPV vaccination for VIN 3 have been reported and are ongoing [Baldwin, 2003; Davidson, 2003; Smyth, 2004]. Distinct from the commercially available preventative vaccines (which only cause a humoral antibody response), the therapeutic vaccines elicit a cellular immune response. The most promising results were reported from a small trial of women with HPV-16-positive VIN 3. Subjects were vaccinated with a mixture of synthetic long-peptides derived from the HPV-16 viral oncoproteins E6 and E7 [Kenter, 2009]. After 12 months there were 6 partial and 9 complete responses in the 19 evaluable subjects, for an overall response rate of 79% (95% CI, 54 to 94%).

Like the imiquimod studies cited above, even partial success with vaccine therapy lends credence to our plan to use the IRX-2 Regimen to treat VIN 3, particularly since the IRX-2 Regimen is devised to overcome the inhibition of local cellular immunity that probably hampers the effectiveness of a systemically administered vaccine therapy.

1.1.5 Immune Deficiency and Immunotherapy in HPV-Mediated Premalignant and Malignant Conditions of the Lower Genital Tract

The rationale for the pursuit of immunotherapy of carcinoma *in situ* of the lower genital tract (such as CIN 3 and VIN 3) with the IRX-2 Regimen stems from a considerable body of evidence which indicates that immunologically competent cells are important host defense mechanisms. Many human cancers, including HPV-related cancers like cervical cancer, are associated with cellular immunodeficiency. Persistent high-risk human papillomavirus (HPV) infection leads to the development of numerous human cancers including cervical, vaginal, vulvar, anal, and an increasing proportion of head and neck cancers that cause significant morbidity and mortality worldwide [Forman, 2012; Tota, 2011]. While genital HPV infections are very common, more than one out of seven HPV-infected women cannot initiate an effective immune response against the virus, and among those that do, clearance is slow and generally takes more than one year [Frazer, 1996; Giuliano, 2002; Stanley, 2007]. To persist in this manner, HPV has evolved mechanisms to escape immune detection, and this persistence is the major risk factor for developing HPV-induced cancers. Previous studies have demonstrated that HPV-mediated suppression of Langerhans cell (LC) immune function is a central mechanism by which HPV evades immune detection [Fahey, 2009; Da Silva, 2015; Fausch, 2005; Woodham, 2015]. This effect is specific to LC, as dendritic cells (DC) are differentially targeted, and thus are activated by HPV. The impact of IRX-2 upon this HPV mediated suppression of LC was investigated after human LC activation by exposure to HPV16 followed by treatment with IRX-2 in vitro [Da Silva, 2016]. The subsequent ability of the LC to induce HPV16-specific T cells was studied. HPV16 alone is not sufficient to induce phenotypic and functional activation of LC, but IRX-2 induces a significant upregulation of antigen presentation and costimulatory molecules, Th1-associated

cytokine release, and chemokine-directed migration of LC pre-exposed to HPV16. Furthermore, LC treated with IRX-2 after HPV16 exposure induced CD8+ T cell responses against specific HLA-A*0201-binding HPV16 T cell epitopes [Da Silva, 2016]. These results clearly suggest that IRX-2 is an attractive immunomodulator for assisting the immune response in eradication of HPV infected cells.

1.1.6 The IRX-2 Regimen for Treatment of CIN and VIN

The concept that subjects with squamous cell cancers and associated dysplastic lesions might benefit from immune stimulation led to the development of the IRX-2 Regimen. The regimen consists of the cell-derived biologic IRX-2 that contains multiple cytokines, plus cyclophosphamide, indomethacin, and zinc with multivitamins. For the treatment of pre-invasive squamous cell neoplasias such as CIN 3 and VIN 3, these four components are administered using an abbreviated schedule, when compared with the IRX-2 Regimen previously studied in subjects with invasive head and neck squamous cell cancers (HNSCC). The Regimen for this protocol consists of an intravenous (IV) infusion of low-dose cyclophosphamide on Day 1, followed by oral indomethacin and oral zinc with multivitamins daily for 21 days, and IRX-2 administered as daily sublesional injections per day for 4 days, as outlined in Section 5. Omeprazole, a proton pump inhibitor, is given concurrently to decrease the likelihood of intolerance of indomethacin.

1.1.7 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 Investigator's Brochure. 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.

1.1.8 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]. Of relevance to the treatment of lower genital tract squamous cell carcinoma *in situ*, similar results were obtained in a later study in cells obtained from subjects with squamous cell cancer of the head and neck (HNSCC) [Schilling, 2013]. Also, in an in vitro study of peripheral blood mononuclear cells obtained from subjects with HNSCC, 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].

The clinical utility of IRX-2 has been validated in a Phase IIa study in subjects with HNSSC (described below in Section 1.2.2). 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 carcinoma *in situ* or cancer can enhance endogenous antigen-specific T-cell responses to the tumor [Naylor, 2010].

1.1.9 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 suppressor 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 suppressor 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. 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 trials 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.

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].

Zinc-containing multivitamins: The importance of zinc in cellular immunity has been described and several reviews are available [Good, 1979; Keen, 1990; Blewett, 2012; Haase, 2014]. Other nutritional deficiencies 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.

1.1.10 Delivery of IRX-2

The sublesional route of administration of IRX-2 takes advantage of the normal afferent and efferent pathways of lymphatic drainage. Typically, 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 CIN 3 and VIN 3, rather than systemically, there is an opportunity to directly mobilize and enhance the function of antigen presenting cells (APCs). The most important APCs in general are the dendritic cells. For the treatment of cervical neoplasia the Langerhans cells (LCs) are the members of the dendritic cell family that are most prominent in the mucosa of the urogenital tract; a variety of dendritic cell types may be important for the treatment of vulvar dysplasia, since those lesions are anatomically at a transition area between vaginal mucosa and skin. Sublesional injection of IRX-2 may also directly activate T cells to proliferate and become cytotoxic lymphocytes. Additionally, sublesional administration may be less toxic since the systemic cytokine drug concentration is much lower.

1.2 Clinical Experience with the IRX-2 Regimen

1.2.1 Phase 1 Trials

The IRX-2 Regimen has been evaluated in two Phase I trials, one in patients with advanced HNSCC and one in patients with early stage cervical cancer.

A Phase 1 trial in subjects with head and neck squamous cell carcinoma (HNSCC) who had progressed after surgery was undertaken to evaluate the clinical and laboratory safety and tolerability of the IRX-2 Regimen. Results of the study, which are presented in the IB and have been published [Freeman, 2010], indicated that the IRX-2 Regimen was well tolerated in these subjects. The reported toxicities were acceptable overall and did not preclude proceeding to additional trials.

An earlier, pilot study of the IRX-2 Regimen was undertaken as neoadjuvant treatment in 10 patients with untreated, resectable cervical cancer [Dueñas-Gonzalez, 2002]. IRX-2 was administered peri-lymphaticaly. All patients were scheduled for hysterectomy on day 21, although three patients opted not to proceed with surgery. Treatment was well tolerated with minor injection related pain and/or bleeding. Gastric symptoms were common, however, leading to substitution of ibuprofen for indomethacin in this study and the addition of omeprazole to subsequent studies of the IRX-2 Regimen. Clinical responses were seen in 5 of the 10 patients (80, 60, 50, 30 and 30%

decreases in the product of longest lesion diameters). Pathological evaluation of the seven resection specimens showed tumor reduction and fragmentation in 5 patients and variable tumor infiltration of lymphocytes, plasma cells, neutrophils, macrophages and eosinophils. Immunohistochemical analysis of the surgical specimens showed an increase in tumor infiltrating CD8+ cells.

1.2.2 Phase 2 Trials in Head & Neck Squamous Cell Carcinoma

Two Phase 2 trials of neoadjuvant IRX-2 have been performed or are underway for the assessment of the IRX-2 Regimen in subjects with locally advanced HNSCC.

The first study (IRX-2 2005 A) was a multicenter 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, have been published [Wolf, 2011; Berinstein, 2012; Whiteside, 2012], and are briefly summarized here.

The second study is a multicenter, international trial entitled "A Randomized Phase 2 Trial of Neoadjuvant and Adjuvant Therapy with the IRX-2 Regimen in Patients with Newly Diagnosed Stage II, III or IVA Squamous Cell Carcinoma of the Oral Cavity" (NCT02609386). This trial designed to include 200 subjects in approximately 50 clinical sites opened for accrual in December, 2015.

1.2.2.1 Study IRX-2 2005-A: Design and Clinical Results

The study objectives were to determine the safety of the IRX-2 Regimen when used as neoadjuvant (preoperative) therapy in a multi-center trial and to evaluate clinical, pathological, and radiographic tumor response and disease-free survival (DFS) and overall survival (OS). Of the 27 subjects, 15 had oral cavity cancer, 8 had oropharyngeal cancer, 1 had hypopharyngeal cancer, and 3 had laryngeal cancer.

The IRX-2 Regimen used for this trial included cyclophosphamide, 300 mg/m^2 on Day 1, 10 days of regional IRX-2 injections between days 4 and 15, and 21 days of oral indomethacin, zinc containing multivitamins and omeprazole.

After 5 years of follow-up, only 7 of the 27 subjects had relapsed. DFS at 1, 2 and 3 years respectively was 72%, 64% and 62%; median DFS has not been reached. OS at 1, 2 and 3 years respectively was 92%, 73%, and 69%; median OS has 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].

1.2.2.2 Study IRX-2 2005-A: Safety Results and Adverse Events

The IRX-2 Regimen was tolerated with minimal toxicity. All subjects completed the regimen and there were no unplanned delays in surgery or difficulties with the planned resections and reconstructions as a result of the immunotherapy regimen.

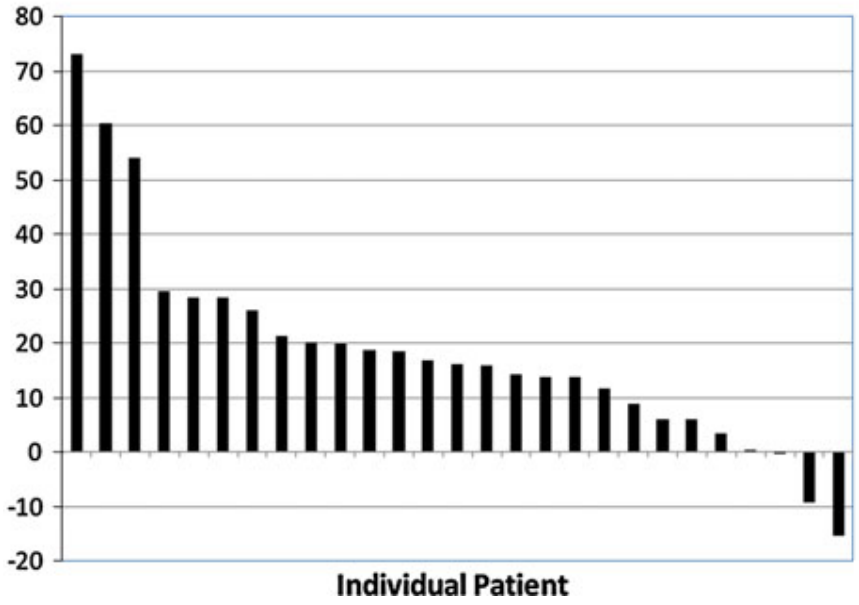
The most common adverse events (AEs) of the IRX-2 regimen (IRX-2, cyclophosphamide, indomethacin, omeprazole, zinc with multivitamins) were headache (30%), injection site pain (22%), nausea (22%), constipation (15%), dizziness (15%), fatigue (11%), aspiration pneumonia (11%), anemia (11%) and myalgia (7%). All were Grade 1-2 except for the aspiration pneumonias

(one Grade 3, one Grade 4); and the aspiration pneumonias were related to the patient's underlying head & neck malignancy. All AEs resolved without sequelae. There were only minor (Grade 1) alterations in post-treatment laboratory values. Of the 8 serious adverse events (SAEs) in 7 subjects reported during treatment and the 30-day post-operative period, none were ascribed to the IRX-2 treatment except for a postoperative wound infection that was possibly related to the study treatment [Wolf, 2011].

1.2.2.3 Study IRX-2 2005-A: Immunologic Results

Pretreatment tumor biopsies and the tumor surgical specimens were characterized for lymphocyte infiltration, necrosis and fibrosis using both hematoxylin and eosin stains and immunohistochemistry [Berinstein, 2012]. 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 1 (change in mean lymphocyte infiltration from the biopsy to the surgical specimen is shown on the y-axis).

Figure 1 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) (p = 0.10) [Brooklyn ImmunoTherapeutics, unpublished observations].

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 1.1.8 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].

1.2.3 Phase 1/2 Cervical Cancer Study

1.2.3.1 Clinical Response

Of the 10 patients treated with perilymphatic, subcutaneous injections of IRX-2 and IRX-2 Regimen, 3 were reported to have a partial response (>50% reduction in the product of the longest perpendicular tumor diameters) and 2 to have minor responses (<50% but >25% reduction in product of tumor diameters) [Dueñas-Gonzalez, 2002]. Regardless of the response observed by these criteria, tumors showed flattening with less bleeding and return of normal pain sensation and epithelial-like appearance. A relationship between clinical response and surgical or pathological findings was not evident.

1.2.3.2 Pathological Response

Five of the seven patients who underwent hysterectomy showed tumor fragmentation with variable patterns of leucocyte infiltration. Immunohistochemical studies showed an increase percentage of CD8+ T cells, and these cells appeared larger and more granular that before treatment. Peripheral blood T cell counts in patients with normal lymphocyte counts pretreatment did not change, but in two patients who had lymphopenia pretreatment, an increase in peripheral blood T cells was noted [Dueñas-Gonzalez, 2002].

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective is to compare the proportion of subjects who achieve a pathologic CR or PR at week 25, based on the resected surgical specimen.

Each cohort (CIN and VIN) will be assessed independently.

2.2 Secondary Objectives

1. To evaluate the toxicity and feasibility of administration of IRX-2 in subjects with confirmed CIN 3 or VIN 3.
2. To evaluate multiple parameters to assess the activity of the IRX-2 Regimen for the treatment of CIN 3 or VIN 3:
    a. The occurrence of clinical CRs or PRs at weeks 6, 13 and 25
    b. Frequency of elimination of HPV in cervical or vulvar tissue using a commercial HPV genotyping assay and viral load determination by quantitative PCR.
    c. Analysis of the immune infiltrates in the resected surgical specimens. Parameters to be evaluated include quantitation of lymphocyte subset infiltration including CD8 and CD4, FOXP3+ cells, evaluation of PDL1 expression, evaluation of state of activation and location of infiltrating regions with respect to dysplasia and adjacent normal tissue. A commercial multiplex IHC assay will assess markers of Langerhans cells (CD1a, E-cadherin) and antigen presenting cell (APC) activation (CD80 and CD86). TCR clonality studies may also be performed.
    d. Immunophenotypic analysis of peripheral blood lymphocytes. Peripheral blood lymphocytes will be evaluated by direct ex-vivo ELISPOT analysis for evidence of induction of γ-Interferon expressing T cells in response to HPV antigens compared to baseline. Changes in naïve, memory, effector and regulatory T cell subset will be analyzed using immunophenotypic flow cytometry.
    e. Frequency of serum antibodies to HPV E6, E7 and L1 proteins by ELISA. Serum cytokine profile will be analyzed by multiplex ELISA at baseline and week 13 (pending availability of the antibodies).
    f. RNA expression profiling of immune-inflammatory markers from post-treatment resected surgical specimens.
    g. Frequency of long term HPV clearance following surgical excision.

3 INVESTIGATIONAL PLAN

3.1 Study Design

This is a Phase 2, open-label study of the IRX-2 Regimen in subjects with CIN 3 or VIN 3. Subjects with CIN 3 will constitute one cohort ("CIN Cohort") and subjects with VIN 3 will constitute a second cohort ("VIN Cohort").

All subjects are to receive standard of care evaluation of lower genital tract dysplasia, including comprehensive colposcopy of the lower genital tract with diagnostic biopsies as clinically indicated. Only women with CIN 3 or VIN 3 who would ordinarily be then managed with an excisional procedure are eligible for this trial (see Section 3.2 for complete inclusion and exclusion criteria). Following the 12 weeks (two cycles) of study treatment all subjects will receive a standard of care excision of the dysplastic lesion involving the cervix or vulva.

For women with cervical dysplasia (the CIN 3 cohort) this usually requires a loop electrosurgical excision procedure (LEEP) of the cervix, with the depth and breadth of the excised tissue based on the geographic extent of dysplasia as determined by the treating physician. A cervical conization procedure (cone biopsy) can also be performed, again at the discretion of the treating physician.

For women in the VIN 3 cohort a wide local excision (partial vulvectomy) will be performed, with the extent of the surgical resection determined by the treating physician based on the extent of the dysplastic lesion. If no lesion is visible on exam, a biopsy will be taken of the original lesion site to confirm complete pathological response.

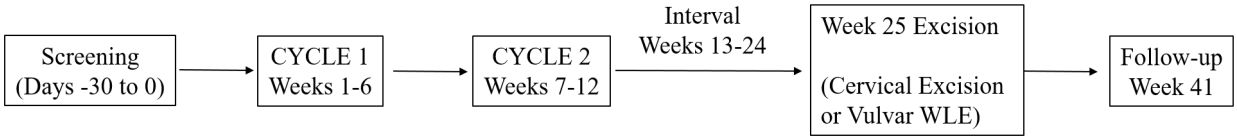
The excisional procedures for subjects in either the CIN or VIN cohort can be done in an office setting, an ambulatory surgery center, or a hospital operating room based on subject and physician preference.

These non-investigational, standard of care colposcopies, biopsies, excisions and related evaluations and supportive care are reasonable and medically necessary for the clinical management of the subject.

Adverse events (AEs) will be assessed daily beginning with the first dose of cyclophosphamide until the week 25 surgical excision. Ongoing AE/SAEs will be reported and monitored until resolution (See Table 2 in Section 5) and classified according to the Common Terminology Criteria for Adverse Events (CTCAE) v4. AEs and SAEs will be tabulated by investigators as definitely, possibly or not related to the study regimen. For further details on AE and SAE data collection, see Section 9.

The flow chart presented in Figure 2 illustrates the study design for both the CIN and VIN cohorts.

Figure 2 Flow Chart of Study Design



3.2 Selection of Study Population

The subject population will consist of 32 subjects with histologically confirmed squamous CIN 3 or VIN 3 who meet the following inclusion and exclusion criteria. Up to 40 subjects may be enrolled to ensure 32 evaluable subjects.

3.2.1 Inclusion Criteria

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

1. Histologically confirmed squamous CIN 3, or VIN 3 (usual type only).
2. Female aged ≥25 years.
3. The subject is either surgically sterile, postmenopausal, or agrees to practice an effective method of birth control as determined by the investigator (to be continued for one year following last dose of study medication), except that subjects with CIN 3 are not permitted to use a cervical cap or diaphragm for contraception.
4. The subject is judged to be in good health based upon the results of a medical history, physical examination, vital signs, and laboratory profile including:

   Hematology:
   - White blood cell >2,500/ mcL (>2.5 x 10^9/L)
   - Absolute neutrophil count >1,000/ mcL (>1 x 10^9/L)
   - Platelet count >75,000/ mcL (>75 x 10^9/L)
   - Hemoglobin ≥8 g/dL (≥80 g/L) (subjects who have received a transfusion or erythropoietin up to one week prior to receiving the first dose of cyclophosphamide are eligible for the study)

   Clotting Time:
   - International normalized ration (INR) or prothrombin time (PT) ≤1.5 x ULN (upper limit of normal)
   - Activated partial thromboplastin time (aPTT) ≤1.5 x ULN

   Renal Function:
   - Serum creatinine ≤1.5 x ULN

   Hepatic function:
   - Total bilirubin ≤2.0 x ULN unless thought to be related to inherited bilirubin conjugation disorder (ie Gilbert's disease)
   - ALT and AST ≤2.0 x ULN
5. The subject is geographically accessible for ongoing follow-up and is committed to comply with the designated visits.

6. The subject is capable of understanding and complying with the protocol and has signed the enrollment informed consent form at screening.

3.2.2 Exclusion Criteria

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

1. For subjects with cervical dysplasia: evidence of atypical glandular cells or adenocarcinoma in situ (ACIS) based on cervical cytology, colposcopy or biopsy.
2. For subjects with either cervical or vulvar squamous dysplasia: evidence of microinvasive squamous carcinoma based on cytology, colposcopy or biopsy.
3. For VIN subjects, those with >2 vulvar lesions.
4. Pregnancy or lactation.
5. Allergy to ciprofloxacin or other quinolones (because ciprofloxacin is used in preparation of IRX-2.)
6. Allergy to indomethacin (a necessary component of the regimen) or to acetylsalicylic acid (aspirin) due to likely allergy cross-reaction.
7. Aldara (imiquimod) for the topical treatment of lower genital tract warts or dysplasia within 3 months of study enrollment.
8. Known to be positive for human immunodeficiency virus-1 (HIV-1) antibody, human immunodeficiency virus-2 (HIV-2) antibody, hepatitis B surface antigen, or hepatitis C virus antibody.
9. Known to have other immunodeficiency diseases, including cellular immunodeficiencies, hypogammaglobulinemia, or dysgammaglobulinemia.
10. Immunotherapy (eg, interferons, tumor necrosis factor, interleukins) or biological response modifiers (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor) or any investigational drug within 3 months of study enrollment.
11. Concurrent treatment with systemic corticosteroids at a dose of ≥5 mg/day of prednisone (or equivalent).
12. Subjects should not take aspirin (except for low-dose aspirin as prescribed for vascular disease) or other non-prescribed, non-steroidal anti-inflammatory agents from start of treatment to surgery.
13. An infectious process or any other significant illness such as an autoimmune disease or advanced age that in the opinion of the investigator would compromise the subject's ability to mount an immune response.
14. Active SARS-CoV2 infection or COVID-19 disease
15. Impaired hepatic, renal or hematological function, evidenced by:
    a. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin >2 times upper limit of normal (ULN),
    b. Serum creatinine >1.5 times ULN, or

16. Clinically significant active cardiovascular disease, including a history of myocardial infarction within the past 6 months, heart failure as defined by New York Heart Association classes III or IV, and/or blood pressure greater than 160/90 mm Hg (1 repeat measure allowed no more than 5 minutes after the first measurement).
17. History of severe allergic reaction to insect bites or stings, or to any biologic pharmaceutical product, including compounds similar to the test article.
18. Any medical contraindications, allergies or previous therapy that would preclude treatment with the components of the IRX-2 Regimen, i.e., cyclophosphamide, indomethacin, zinc-containing multivitamins or omeprazole.
19. Donation or loss of >450 mL of blood or plasma within 30 days of start of treatment.

3.3 Assessment of Dysplastic Lesion and Surgical Resectability

For women with CIN, a colposcopic examination will be performed after application of 5% acetic acid to the cervix. The site (based on a 1-12 o'clock system) and appearance of each suspicious lesion (colpsocopic features suggestive of CIN 3 include acetowhitening and the presence of specific vascular patterns such as punctate vessels or mosaicism) will be documented. A rebiopsy will not be performed unless the visualized lesions have features suggestive of invasive cancer, such as presence of a gross lesion. A determination will be made as to the "adequacy" of the colpsocopic examination (whether the entirety of each lesion can be seen and as to whether the entirety of the squamocolumnar junction can be seen). A photograph will be taken with the attached camera at each colposcopic evaluation. At the prescribed time, an excisional procedure (either a large loop excision of the transformation zone for women who have had an adequate colposcopy and negative ECC) or a conization biopsy (to be performed either via LEEP technique or via the cold knife technique, depending on the judgment of the surgeon as to whether the patient requires general anesthesia for the procedure for women with a positive ECC or in whom the colposcopy is not adequate) will be performed.

For women with VIN 3, the site of all lesions based on which anatomical portion of the vulva the lesion is involving, as well as the size of each lesion, measured with a ruler will be recorded. The vulva will be photographed at each vulvar examination. At the proscribed time, either a wide local excision or biopsy of each residual lesion followed by laser ablation will be performed, depending on the number and sites of residual lesions. If a lesion is no longer visible at a site where a lesion was seen at study entry, a punch biopsy will be taken of the area to assess for pathologic complete response.

3.4 Termination of Study Treatment

The investigator can 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 Brooklyn ImmunoTherapeutics within 24 hours of the event. Regardless of the reason for study treatment discontinuation, the investigator must complete the Study Completion Form in the Case Report Form (CRF), specifying the reason. Subjects should continue follow-up visits and be monitored for recurrence and survival, unless the subject withdraws consent for follow-up.

NOTE: Subjects may experience changes in injection sites 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 malignant transformation or progression.

3.5 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.

An attempt will be made, unless consent for follow-up is specifically withdrawn, to follow all subjects per study protocol, with emphasis on ensuring the subject does undergo the necessary surgical excision of the dysplastic lesion (e.g. cervical LEEP or cold knife conization or vulvar WLE).

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

3.6 Medical Monitoring

3.6.1 Medical Monitors

The Medical Monitors are physicians trained in the conduct of clinical trials who are available to the clinical investigators for discussion of questions regarding the inclusion/exclusion criteria and any other medical issues that may arise.

3.6.2 Principal Investigator(s)

The Study Principal Investigator or his designee should be contacted to discuss questions related to enrollment, subject assessment, surgical resectability, and optimal technique for the cervical LEEP or vulvar WLE, and any other details of the study.

Principal Investigator:	Lynda Roman, MD
Contact information:	(213) 919-0209 (Pager)

3.7 Data Safety Monitoring

The Study Team (PI, Co-PI's, and Study Statistician) will meet at least monthly and as needed by teleconference to review the progress of the trial as well as all AE's. Minutes will be taken at each meeting and circulated to the Study Team.

Should a decision be made that toxicities and adverse events due to the IRX-2 regimen are too frequent or unexpectedly severe, the Study Team will propose modifications to the regimen beyond those specified in Section 5.2.9 and if appropriate, the protocol will be formally amended. Section 8.1 describes the technical aspects of the proposed safety monitoring.

The toxicities, study compliance and progress, will be reviewed every 6 months by the USC/Norris

Comprehensive Cancer Center DSMB. This DSMB will review the actions and modifications taken by the Study Team in response to issues that have arisen during the conduct of the trial.

4 STUDY TREATMENTS

4.1 Study Design

Subjects who meet all eligibility criteria will receive the IRX Regimen.

4.2 IRX-2 Regimen

In this trial, the IRX-2 Regimen (as shown in Table 1, below) will include cyclophosphamide 3 days before the start of IRX-2, IRX-2 daily x 4 consecutive days, and indomethacin, zinc with multivitamins, and a proton pump inhibitor for the first 21 days of each six week cycle.

All treatments will be repeated beginning on Week 7, for a total of two cycles.

Table 1 IRX-2 Regimen

Agent	Dose	Route of Administration	Treatment Days (each cycle) Cycle = 6 weeks
Cyclophosphamide	300 mg/m^2	IV	Day 1
IRX-2	230 units daily (in 2 ml) in 4 divided doses: 57.5 units (1/2 ml) in each quadrant of the cervix or affected areas of the vulva	Submucosal injection in the cervix; subcutaneous for vulvar lesions	Days 4-7
Indomethacin	25 mg TID	Oral	Days 1-21
Zinc with multivitamins	1 tab daily	Oral	Days 1-21
Omeprazole	20 mg daily	Oral	Days 1-21

All IRX-2 preparations will be prepared by the pharmacist or designated staff. The IRX-2 will be transferred from their sterile vials to syringes provided by Brooklyn ImmunoTherapeutics.

4.3 Route of Administration

For cervical dysplasia: IRX-2 will be administered submucosally in the cervix with four divided doses into each quadrant of the cervix, irrespective of location of the CIN 3 lesion.

In women with CIN 3, 2.0 cc of study drug will be drawn into one 3 cc syringe, which will be attached to a 22 gauge spinal needle (9cm length). 0.5 cc of study drug will be injected into the submucosa of each of the 4 quadrants of the cervix.

For vulvar dysplasia: In women with VIN 3, 2.0 cc of study drug will be drawn into a 3 cc syringe which will then be attached to a 25 gauge needle. 0.5 cc of study drug will be injected at 12, 3, 6 and 9 o'clock at the borders of a single lesion, into the subcutaneous tissue beneath the lesion. For

>1 lesion, doses shall be divided at clinician discretion based on the geometry and size of the dysplastic lesions.

4.4 Regimen Medications

4.4.1 IRX-2

IRX-2 is supplied as a pale yellow, sterile liquid for subcutaneous injection in 2.0 mL single-dose vials. IRX-2 must be stored in a secure area and maintained under labeled storage conditions at a range of −15º C to –50º C. All study supply containers will be appropriately labeled to identify study number, kit numbers, lot number, and product identity. Study syringes for subcutaneous study drug administration will be disposed of in accordance with biosafety procedures. Empty study drug vials will be retained for accountability and may be disposed of on-site following drug reconciliation.

4.4.2 Other Medications

Cyclophosphamide, indomethacin, zinc with multivitamins and omeprazole shall be sourced locally for each study subject.

4.5 Method of Assigning Subject Numbers

After a subject has signed the informed consent form and has been deemed eligible for enrollment, the subject will be assigned a 3-digit enrollment numbering (starting with 001) such that all subjects are given consecutive identification numbers in successive order of enrollment.

4.6 Treatment Compliance

The investigator or other study personnel will administer study drug injections to ensure treatment compliance. Subjects should be asked each day that they are seen about compliance with oral medications and compliance (or lack thereof) documented in the clinical record.

4.7 Prohibited, Prior and Concomitant Treatments

Subjects, from start of treatment to completion of Cycle 2, should **not** take aspirin (except for low-dose aspirin as prescribed for vascular disease) 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 start of treatment to the 25th week excisional procedure will be recorded. Medications given to treat a reported AE or SAE will continue to be recorded until the AE/SAE resolves.

5 STUDY PROCEDURES AND SCHEDULE

5.1 Study Schedule

The Schedule of Study Procedures is outlined in Table 2 and **Table 3**.

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” and 2 weeks for tests indicated in months is permitted.

See also Appendix 4 for the optimal schedule for investigators at USC. For subjects at that institution, the "Day 1" cyclophosphamide is best infused on a Thursday or Friday; the subsequent IRX-2 injections are performed on the subsequent Monday through Thursday or Tuesday to Friday (Days 4, 5, 6, 7 or Days 5, 6, 7, 8).

Table 2 Schedule of Events

Study Procedure	Screening (Baseline)	Treatment Cycles																				Pre-procedure visit	Surgical Resection (LEEP or wide local excision)	Post-Op Check[i]	4-month post-op
		Cycle 1: 1st 6-weeks									Cycle 2: 2nd 6-weeks														
	Days −30 to 0	Day(s) of Cycle 1									Days(s) of Cycle 2														
		1	2	3	4	5	6	7	>8		1	2	3	4	5	6	7	>8				Week 21	Week 25	Weeks 26+	Week 41
Day(s) of Protocol		1	2	3	4	5	6	7	8-21	22-42	43	44	45	46	47	48	49	50-63	64-84	91	Day 147 ± 14 days	Day 175 ± 7 days[g]		Day 290 ± 14 days
Informed consent	X																								
Study Drug Administration																									
Cyclophosphamide IV[a]		X									X														
Indomethacin, omeprazole, zinc with multivitamins[b]		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X							
IRX-2 injection[b]					X	X	X	X						X	X	X	X								
Procedures & Evaluations																									
Complete History & Physical Exam	X										X									X	X				
HIV-1, HIV-2, HBV, HCV	X																								
SARS-CoV2		X[c]									X[c]														
Focused pelvic exam																							X	X[j]	X
Injection Site Check					X			X						X			X			X	X				
ECOG Performance Status	X	X						X			X						X			X	X			X	X
AEs and SAEs[d]		X			X	X	X	X			X			X	X	X	X			X	X			X	X
Concomitant Medications	X	X			X	X	X	X			X			X	X	X	X			X	X			X	X
Chemistry, CBC, PT/INR	X	X[e]									X[e]									X[e]	X[e]				
Blood work for investigational studies	X[e]	X[e]									X[e]									X[e]	X[e]				
Blood for PK analysis[h]					X	X	X	X						X	X	X	X								
Pregnancy Test[f]	X	X[f]									X[f]									X[f]	X[f]				

Colposcopy & cervix photography (CIN patients)	X										X									X	X			
Cervical inspection & photography (CIN patients)					X[L]			X[L]						X[L]			X[L]							
Cervical cytology (pap smear/ECC)(CIN patients)																				X	X			X
Vulvar inspection & photography (VIN patients)	X				X[L]			X[L]			X			X[L]			X[L]			X	X			
Cervical or vulvar swab for HPV assay (research)	X																			X	X			X
Cervical or vulvar biopsy	X[k]																							
Cervical LEEP excision or cone biopsy or vulvar excision or biopsy																						X		

Footnotes for Table 2:

a. Dexamethasone or other steroids should not be used as an anti-emetic with the cyclophosphamide. Steroid administration may inhibit IRX-2 Regimen stimulation of an immune response. The prescribed dose is significantly less than used for conventional chemotherapy and thus steroids will not be needed. Suggested antiemetic regimen would include a serotonin (5-HT3) antagonist and lorazepam.
b. Subjects will receive injection of either study agent IRX-2 daily on Days 4, 5, 6, 7 or Days 5, 6, 7, 8. The indomethacin, omeprazole, zinc with multivitamins are administered for the first 3 weeks per cycle, on Days 1-21. For cycle 2 [Week 7] the comparable "Days" are shown in the table.
c. SARS-CoV2 virus test required within 2-4 days prior to start of Cycle 1 and Cycle 2. If positive, treatment will be held.
d. Adverse events (AEs) and serious adverse events (SAEs) are measured from day of Cycle 1 Day 1 until Week 25 of the study. Ongoing AE/SAEs will be reported and monitored until resolution.
e. Ten ACD yellow top blood tubes (85 mL) and one red top serum tube should be drawn for investigational studies. Blood work can be done up to seven days prior to the specified day. Blood draws do not need to be repeated on indicated day if drawn within the previous 7 days.
f. Pregnancy must be ruled out, or post-menopausal status verified, at study enrollment. Fertile subjects will need effective contraception during the study and for 1 year following last dose of study medication; investigator to repeat pregnancy tests during the study if needed for verification. A blood pregnancy test should be used at screening, week 7 and week 25. A urine pregnancy test can be performed on Cycle 1 day 1 visit, and at week 13 safety visit.
g. The "Week 25" lesion excision is to be scheduled for Day 175, +/- 7 days.
h. One red top serum tube (5 mL) required at each of the following time points: Day 4 pre-injection, 30 minutes, 1 hour, 2 hours post-injection; Day 5 pre-injection; Day 6 pre-injection; Day 7 pre-injection, and then 30 minutes, 1 hour, 2 hours post-injection. All time points allow +/- 5 minutes.
i. Post-op check to be performed 1 to 8 weeks post-surgery; can occur by phone or office visit.
j. If seen for office visit.
k. If previous biopsy not done within past 60 days, or if slides/block from this are not available, or at discretion of investigator based on clinical impression.
l. Photography to be performed prior to injection of study drug.

Table 3: Schedule of Virologic and Immune Monitoring (All Subjects) Based on Blood, Swab or Tissue Samples

Study Procedure	Screening (Baseline)	Treatment Cycles		Safety visit	Surgical Excision	4-month follow-up
		Cycle 1	Cycle 2	Week 13	Week 25	Week 41
	Days -30 to 0	Day 1	Day 43 (Cycle 2, Day 1)	Day 91	Day 175 ± 7 days	Day 290 ± 14 days
Presence or Absence of HPV[c]	X				X	X
HPV Genotyping[c]	X				X	X
Flow Cytometry[c]	X	X[a,b]	X[b]	X[b]	X[b]	
ELISPOT[c]	X	X[a,b]	X[b]	X[b]	X[b]	
ELISA[c]	X	X[a,b]	X[b]	X[b]	X[b]	
Phenotypic analysis of lymphocytic infiltrate[d]	X				X	

a. Cycle 1, Day 1 labs do not need to be repeated if the Screening (Baseline) labs were drawn within four days. For subject convenience, investigator should synchronize these phlebotomies with the blood draws specified in Table 1.

b. Blood work for ELISpot, ELISA and flow cytometry can be collected up to four days prior to the specified day (e.g. labs can be obtained on M-T-W-Th before a Friday treatment day).

c. HPV genotyping, flow cytometry, ELISPOT, and ELISA assays will be performed in the Norris Comprehensive Cancer Center Immune Monitoring Core laboratory.

d. Tissue immunohistochemistry and PK studies will be done by Brooklyn ImmunoTherapeutics via a third party.

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.

The following screening evaluations are to be performed within 30 days prior to beginning treatment:

- History of present illness, including recording of prior cytology and histology results, and collection of corresponding laboratory reports with subject identifiers and accession numbers to allow retrieval of archived specimens for comparative studies.
- Medical history, including assessment of all entry and exclusionary criteria, demographics (age, sex, race, menstrual status, weight, height), history of hypersensitivity (drug allergies), general medical history, and assessment of all current symptoms including severity.
- History and current use of tobacco and/or alcohol.
- Determination of ECOG performance status (Appendix 3).
- Concomitant medication review.
- General physical examination, including vital signs (blood pressure and pulse).
- Detailed pelvic examination, including:
    - Cervical swab for HPV status and genotyping for investigational studies;
    - Colposcopy of the lower genital tract and detailed description of the dysplastic lesion(s);
    - Photography of the dysplastic lesion(s)
    - Biopsy of dysplastic lesion(s), if not done at time of initial diagnosis; if a biopsy has been done previously within the past 60 days, it is not necessary, nor desirable, to repeat it.
- 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.
- Pregnancy test (serum) <OR> confirmation subject is post-menopausal (absence of menses for > six months and LH or FSH in menopausal range).
- Collect additional blood samples for investigational correlative studies, including assessment of the immunogenicity of IRX-2.

All of the above screening evaluations, except for the collection of blood samples for investigational correlative studies, are reasonable and medically necessary for the direct clinical management of the subject, and thus should be appropriate for reimbursement by insurance providers [Martin, 2014].

5.2.2 Cycle 1, Day 1

The following procedures will be completed at this visit:

- Review documentation of history, physical exam, and laboratory information obtained during screening assessment.
- Review results of SARS-CoV2 viral RNA test administered within the prior 2-4 days. A positive test will result in delay of study drug administration until infection clears.
- Interval history and assessment of all current symptoms (AEs) including severity.
- ECOG performance status.
- Record concomitant medications.
- Repeat the following laboratory assessments if more than 3 weeks since screening values obtained.
    - Hematology: CBC, differential, platelet count, PT and PTT.
    - Serum chemistry: serum albumin, total protein, serum bilirubin, LDH, ALT/SGPT, AST/SGOT, creatinine, BUN, glucose, and electrolytes.
- Verification premenopausal subjects are not pregnant (by history and/or laboratory studies at discretion of investigator. Urine pregnancy test is acceptable).
- Collect blood samples for investigational correlative studies, including assessment of the immunogenicity of IRX-2 (if not collected at screening visit).
- Administer the following medications:
    - Infusion of cyclophosphamide, 300 mg/m^2, over a one hour period.
    - Steroids must not be administered as anti-emetic 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, approx. q 8 hours [TID]) to be taken orally after meals from Day 1 through Day 21.
    - Begin zinc (24 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 Cycle 1, Days 4 to 7 or Days 5 to 8

On Treatment Days 4 to 7 (or Days 5 to 8), the investigational pharmacist will supply the clinical investigators with the pre-filled syringes containing 2.0 ml of IRX-2 solution. The solution will be administered by injection, as follows:

- Total daily dose of the IRX-2 is 230 units, contained in 2 ml of solution.
- For the CIN 3 cohort: The solution should be administered in four divided doses into the cervical mucosa of each quadrant of the cervix (0.5 ml, or 57.5 U, per injection).
- For the VIN 3 cohort: The solution should be administered into the subcutaneous tissue beneath the dysplastic lesion(s) in divided doses. The number and distribution of the injections is deferred to the investigator based on the size and geometry of the vulvar lesion. The investigator will record this information, aided by diagrams and/or photographs.
- On each injection day, subjects will be monitored for at least 15 minutes after the injections for signs or symptoms of any reaction.

On the Day 4 visit (the first day of solution injections), the following evaluations will be performed and documented:

- Interval history and assessment of all current symptoms including severity (AEs);
    - Irrespective of scheduled days for AE assessment, any information regarding AEs received by clinicians caring for the subject will be documented and the study site personnel informed.
- Record concomitant medications;
- Evaluation of injection sites;
- Photography of the lesion site obtained before the injection of IRX-2;
- Blood will be drawn pre-injection, followed by 30 minutes, 1 hour, and 2 hours after injection for pharmacokinetic analysis. All time points allow +/- 5 minutes.

On the Day 5 and Day 6 visits (the second and third day of solution injections), the following evaluations will be performed and documented:

- Interval history and assessment of all current symptoms including severity (AEs);
    - Irrespective of scheduled days for AE assessment, any information regarding AEs received by clinicians caring for the subject will be documented and the study site personnel informed.
- Record concomitant medications;
- Evaluation of injection sites;
- Blood will be drawn pre-injection for pharmacokinetic analysis

On the Day 7 visit (the fourth day of solution injections), the following evaluations will be performed and documented:

- Interval history and assessment of all current symptoms including severity (AEs);
    - Irrespective of scheduled days for AE assessment, any information regarding AEs received by clinicians caring for the subject will be documented and the study site personnel informed.
- Record concomitant medications;
- Evaluation of injection sites;
- Photography of the lesion site obtained before the injection of IRX-2;
- Blood will be drawn pre-injection, followed by 30 minutes, 1 hour, and 2 hours after injection for pharmacokinetic analysis. All time points allow +/- 5 minutes.
- Any missed doses due to reasons other than toxicity should be administered as soon as possible within 10 days from the first injection, not to exceed one injection dose per day. If ten days has elapsed from initial IRX-2 injection, patient should continue being monitored until the next scheduled study visit. Missed doses should be documented on case report forms.

5.2.4 Cycle 1, Days 8 to 21

Subjects continue the indomethacin, omeprazole, zinc with multivitamins regimen.

5.2.5 Cycle 2, Day 1 (Beginning of Week 7; Day 43 of Protocol)

Subjects return at the start of Week 7 to begin the second of 2 cycles of treatment. Day 43 is almost identical to Day 1, as detailed below:

- Review documentation of history, physical exam, and laboratory information obtained during screening assessment and during the first cycle of treatment.
- Review results of SARS-CoV2 viral RNA test administered within the prior 2-4 days. A positive test will result in delay of study drug administration until infection clears.
- Interval history and assessment of all current symptoms (AEs) including severity.
- ECOG performance status.
- Record concomitant medications.
- Brief general physical examination including weight and vital signs (blood pressure and pulse).
- Clinical laboratory assessments:
    - Hematology: CBC, differential, platelet count, PT and PTT.
    - Serum chemistry: serum albumin, total protein, serum bilirubin, LDH, ALT/SGPT, AST/SGOT, creatinine, BUN, glucose, and electrolytes.
- Pregnancy test (serum or urine) <OR> confirmation subject is post-menopausal (absence of menses for > six months and LH or FSH in menopausal range).

- Collect blood samples for investigational correlative studies, including assessment of the immunogenicity of IRX-2.
- Administer the following medications:
  - Infusion of cyclophosphamide, 300 mg/m^2, over a one hour period.
  - Steroids must not be administered as anti-emetic 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, approx. q 8 hours (TID)) to be taken orally after meals from Day 43 through Day 49 (Cycle 2, days 1-21).
  - Begin zinc (24 mg, once daily) with multivitamins to be taken orally from Day 43 through Day 49 (Cycle 2, days 1-21).
  - Begin omeprazole (20 mg, once daily) to be taken orally after meals from Day 43 through Day 49 (Cycle 2, days 1-21).

5.2.6 Cycle 2, Days 4 to 7 (Continuation of Week 7, or days 46 to 49 of Protocol)

During these days the subjects receive the second series of solution injections.

Procedures are identical to Cycle 1, Days 4-7 detailed in Section 5.2.3 (above).

5.2.7 Cycle 2, Days 8 to 21 (Days 50 to 63 of Protocol)

Subjects continue the indomethacin, omeprazole, zinc with multivitamins regimen.

5.2.8 Management of Toxicity Related to IRX-2 Regimen.

In the event of Grade 3 or higher toxicity, no further IRX-2 should be injected and indomethacin should be discontinued for that Cycle. Should the toxicity occur during Cycle 1 and if the subject has fully recovered, Cycle 2 may be given at full or reduced doses as specified below. In the event of Grade 2 toxicity, treatment should be held until stable and then resumed per protocol, but if treatment must be delayed for more than 7 days that Cycle should be discontinued, except that omeprazole and zinc with multivitamins can be continued at the discretion of the investigator.

For subjects with toxicity most likely related to indomethacin (e.g. gastrointestinal intolerance), the indomethacin may be discontinued or the dose reduced and the rest of the protocol treatment continued.

For subjects with toxicity most likely related to cyclophosphamide, including myelosuppression or hematuria, the Cycle 2 dose should be reduced by 50%. For cyclophosphamide related unacceptable nausea or for emesis, the antiemetic regimen should be enhanced by adding a neurokinin-1 antagonist or olanzapine, but corticosteroids should not be administered.

For subjects with Grade 2 or greater local toxicity most likely related to the injections of IRX-2, the details of administration, as specified in Section 4.3, may be modified at the discretion of the investigator, e.g. by increasing or decreasing the number of injections or the volume per injection (not to exceed 2 mL), or the total dose of IRX-2 may be decreased by 50%.

5.2.9 Week 13: Safety Assessment

Subjects will return on week 13 for a safety assessment. The purpose of this visit will be to assess the well-being of the subject including performance status, adverse event profile and injection site reactions. In addition, the dysplastic lesion will be assessed either by direct visual inspection and photography or by colposcopy. The status of the lesion will be determined and regression will be documented. If there is evidence of progression consideration will be given to performing the surgical excision at this time (see Sections 5.2.11 and 5.2.12). Exploratory assays from the blood will be performed at this timepoint.

- Review documentation of history, and laboratory information obtained while on study.
- Complete physical.
- Assessment of all current symptoms (AEs) including severity.
- ECOG performance status.
- Record concomitant medications.
- Pelvic examination with colposcopy (CIN patients) and photographs of the lesion (CIN and VIN patients).
- Evaluation of injection sites.
- Cervical cytology (pap smear/ECC) for CIN patients.
- Clinical laboratory assessments:
    - Hematology: CBC, differential, platelet count, PT and PTT.
    - Serum chemistry: serum albumin, total protein, serum bilirubin, LDH, ALT/SGPT, AST/SGOT, creatinine, BUN, glucose, and electrolytes.
- Pregnancy test (serum or urine) <OR> confirmation subject is post-menopausal (absence of menses for > six months and LH or FSH in menopausal range).
- Collect blood samples for investigational correlative studies, including assessment of the immunogenicity of IRX-2.
- Cervical or vulvar swab for HPV status and genotyping for investigational studies.

5.2.10 Week 21-23: Pre-Surgical Assessment

Subjects return between Week 21-23 for pre-surgical assessment, counseling and surgical scheduling for excision of the dysplastic lesion. As detailed in Section 7, for some subjects the excision will be an office procedure – either a cervical LLETZ for the CIN 3 cohort or a vulvar WLE for the VIN 3 cohort. Some procedures will require surgery in an outpatient surgical center or hospital-based operating room, depending on subject preference and clinical considerations deferred to the managing clinician; those subjects will likely have a separate “pre-operative visit” at week 21-23, or based on local institutional policies for outpatient surgery.

Regardless, the assessments listed below are to be done no earlier than four weeks prior to the excisional procedure; e.g. from -28 to 0 days before excision of the dysplastic lesion.

- Review documentation of history, and laboratory information obtained while on study.
- Assessment of all current symptoms (AEs) including severity.
- ECOG performance status.
- Record concomitant medications.
- Complete physical.
- Pelvic examination with colposcopy (CIN patients) and photographs of the lesion (CIN and VIN patients).
- Evaluation of injection sites.
- Cervical cytology (pap smear/ECC) for CIN patients.
- Pregnancy test (serum or urine) <OR> confirmation subject is post-menopausal (absence of menses for > six months and LH or FSH in menopausal range).
- Clinical laboratory assessments:
  - Hematology: CBC, differential, platelet count, PT and PTT
  - Serum chemistry: serum albumin, total protein, serum bilirubin, LDH, ALT/SGPT, AST/SGOT, creatinine, BUN, glucose, and electrolytes.
- Collect blood samples for investigational correlative studies, including assessment of the immunogenicity of IRX-2.
- Cervical or vulvar swab for HPV status and genotyping.

All of the above pre-surgical evaluations, except for the collection of blood samples and cervical swab for investigational correlative studies, are reasonable and medically necessary for the direct clinical management of the subject, and thus should be appropriate for reimbursement by insurance providers [Martin, 2014].

5.2.11 Surgical Excision of the Dysplastic Lesion: Week 25 (Day 175 of Protocol)

All subjects will undergo excision of their dysplastic lesion following the usual and customary care guidelines presented in Section 7.

Surgery is to take place on or around Day 175 (Week 25) of the protocol, adjusted if needed for any treatment delays allowed for toxicity (See Section 5.2.9) or minor changes in schedule within the acceptable parameters (See Section 5.1). Regardless, surgical excision should occur no later than 7 months since starting treatment to avoid unacceptable delay in definitive surgical management of the subject's dysplastic lesion.

For CIN3 patients, in cases where no identifiable lesion is seen on colposcopy at the pre-procedure visit, the surgical approach (LLETZ, versus cone biopsy via either LEEP or cold knife technique) will be the same as that which the subject would have received at baseline to rule out any occult dysplasia, particularly in the endocervix.

For VIN3 patients with no visible lesion at the end of the study period, the extent of surgical excision will be different from that of VIN3 at baseline. Instead of a WLE, a punch biopsy will be performed in the location of the original lesion. If the VIN lesion is smaller in diameter compared

to baseline, these lesions will be removed with a surgical approach appropriate to the size and location of the lesion at week 25, at the discretion of the treating physician.

The surgical specimen will be processed as detailed in the Laboratory Manual. Conventional histologic assessment will be done and a pathology report generated for the subject's medical record following routine practice at the institution. Routine histologic assessment that guides clinical management of the subject will take priority over the investigational studies.

The surgical excision of the dysplastic lesions and routine pathology assessment are reasonable and medically necessary for the direct clinical management of the subject, and thus should be appropriate for reimbursement by insurance providers [Martin, 2014]. Expenses for the investigational studies performed using the excised tissue will be borne by Brooklyn ImmunoTherapeutics.

5.2.12 Short-Term Follow-Up Schedule

Consistent with usual and customary care, all subjects will have at least a telephone contact from the clinical investigators 1 to 8 weeks after the excisional procedure to inquire about post-procedure recovery, inform the subject of the final pathology report, and discuss plans for long-term surveillance of the subject's lower genital tract dysplasia. Based upon clinician or subject preference, this post-procedure assessment can be performed as part of an office visit.

The post-procedure assessment, whether done by phone or during an office visit, will include:

- Interval history and assessment of all current symptoms including severity (AEs)
- Record concomitant medications.
- ECOG performance status.
- And IF subject is seen for an office visit:
    - Detailed pelvic examination, including:
        - Inspection of surgical site

Subjects should be seen more frequently for management of post-procedure complications, other medical problems, or regimen-related toxicities at the discretion of the managing clinician. Information from these additional encounters will be collected on CRFs and reported to the study team.

Routine post-procedure care, including office visits advised by the clinician, and care of any expected complications related to the surgical procedure itself, is appropriate for reimbursement by insurance providers [Martin, 2014]. Other study-related visits, such as photography at follow-up office visits, as well as care required for the management of study-related AEs, will be borne by Brooklyn ImmunoTherapeutics.

5.2.13 Four-month follow-up surveillance

Subjects return four months post excisional procedure or biopsy to assess HPV status post treatment. The assessments listed below are to be done no earlier than 16 weeks from the excisional procedure.

- Focused pelvic exam

- Assessment of all current symptoms (AEs) including severity.
- ECOG performance status.
- Record concomitant medications.
- Cervical cytology (pap smear) for CIN patients.
- Cervical or vulvar swab for HPV status and genotyping.

Routine follow-up care, including office visits advised by the clinician, and care of any expected complications related to the surgical procedure itself, is appropriate for reimbursement by insurance providers [Martin, 2014]. Study-related procedures, such as HPV swab at follow-up office visits, as well as care required for the management of study-related AEs, will be borne by Brooklyn ImmunoTherapeutics.

5.2.14 Longer-Term Follow-Up

Active surveillance of subjects will cease after a) all treatment-related toxicities have resolved and b) completion of the 4-month post-excision assessment detailed in Section 5.2.13.

If all toxicities related to participation in the study have not resolved by the final office visit post surgical excision, investigators may obtain longer-term follow-up information from the subjects' medical records related to these delayed complications or adverse events related to the study, as well as any history or laboratory evidence of lower genital tract dysplasias or cancers. This additional information will be collected in a HIPAA-compliant fashion with informed consent of the subject and will be narrowly focused for only this study of IRX-2 treatment of lower genital tract dysplasia.

6 STUDY ASSESSMENTS

6.1 Endpoint Assessments

6.1.1 Primary Endpoint – Pathologic Response Rate

The primary endpoint is the response of CIN 3 or VIN 3 lesions to the IRX-2 regimen as determined by histologic examination of the Week 25 surgical excision specimen. Complete response is defined as a reduction to no CIN or VIN. The number of subjects with lesions that change to a lower grade of dysplasia (CIN/VIN 1) will also be quantified, as well as subjects with evidence of persistent CIN/VIN 3 or progression to invasive cancer.

Pathologic Complete response	Disappearance of any dysplasia
Pathologic Partial response	Regression of CIN3 to CIN1 in cervical cases; and VIN3 to VIN1 I in vulvar cases
Pathologic Non-Responder	Stable high grade cervical dysplasia (CIN2 or CIN3) or stable high grade vulvar dysplasia (VIN2 or VIN3)
Pathologic Progression	Evidence of invasive carcinoma

Histologic evaluation of both the initial diagnostic biopsies and the subsequent surgical excisions (For CIN3: cervical LEEP or Conization; For VIN3: vulvar WLE or biopsy of original lesion site) will be performed and reported to the clinicians caring for each subject by the pathology laboratory. Clinical decisions will be based on this information.

For the purpose of the study, a pathologist with specialty expertise in the assessment of lower genital tract dysplasia will also evaluate the specimen. In the event of a clinically significant diagnostic discrepancy between different pathologists, the principal investigator and the clinician caring for the subject will be notified, steps taken to resolve the discrepancy, the subject informed of the final diagnostic decision, and her clinical management altered if necessary.

The gynecologic pathologist selected for this study at USC is Dr. Saloni Walia (USC Department of Pathology). Gynecological pathologists from other participating sites will be selected by the institutions Principal Investigator. Entry biopsy slides and final excisional procedure pathology slides obtained at other clinical sites will be reviewed by USC's pathologists for confirmation of final diagnosis.

6.1.2 Secondary Endpoints

6.1.2.1 Toxicity and Feasibility

Toxicity of the IRX-2 Regimen overall, and specifically for the IRX-2 injection, will be assessed by the incidence and severity of AEs, SAE, as classified and graded according to the current version of the CTCAE v4, with one modification: attribution of AEs will be coded as definitely,

possibly, and not related to treatment (per Section 9.1.2). All subjects will be assessed for AEs as detailed on the Study Schedules (Section 5.1) and evaluated for severity.

Feasibility for each patient will be determined if she (a) has received the 2 full cycles of treatment (specifically all 4 doses of IRX-2 in each cycle) and (b) can undergo surgical resection within the planned time window.

6.1.2.2 Clinical CR or PR at Weeks 6, 13 and 25

At the time of the planned colposcopies (and photographs) at weeks 6, 13, and 25, the lesions sizes will be evaluated. Clinical complete response is no evidence of CIN or VIN visually by colposcopy and documented by the photographs. Clinical partial response is defined as a reduction in diameter of the lesion by 50% or greater.

6.1.2.3 HPV Status

HPV status will be assessed at baseline, at week 13 (at the time of planned colposcopy), at week 25 (at the time of surgical resection), and at 4 months post excision or biopsy. HPV status will be classified as positive if HPV virus is detected by PCR or negative if it is not detected. The log reduction in the viral load will be recorded.

6.1.2.4 Immunologic Studies

Multiple parameters will also be studied to assess the immunologic activity of the IRX-2 Regimen for the treatment of CIN 3 or VIN 3, as detailed below. These studies are investigational and costs will be borne by Brooklyn ImmunoTherapeutics. These will include assessments of serologic response to HPV by ELISA, evidence of an antigen-specific T cell response by ELISPOT and changes in peripheral blood lymphocyte subsets and state of activation by flow cytometry. In addition, the development of anti-drug antibody (ADA) immune responses to the active components of IRX-2 will be measured in blood samples collected at pre-dose of 1st cycle (day 1), week 1-2, pre-dose of 2nd cycle (week 6), week 13, and week 25. Blood samples collected at the time of injections (days 4-7 of each cycle) will be used for pharmacokinetic analysis.

6.1.2.5 Characterization of the Immune Infiltrate within the Surgical Specimens

Analysis of the immune infiltrates in the pre-treatment tumor biopsies and the post-treatment excisional specimens will be determined and compared where sufficient biopsy/excisional material is available. Parameters to be evaluated include:

- Lymphocyte infiltration within the center of the tumor and at the tumor edge or margins.
- Lymphocyte subsets 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 and APCs. IHC assay will also assess markers of Langerhans cells (CD1a, e-cadherin) and antigen presenting cell (APC) activation (CD80 and CD86). Other immune subsets and activation markers may be evaluated. Multiplex IHC assays may be used.
- Gene expression signatures within the dysplastic tissue may also be performed.

- Lymphocyte infiltrates and changes after treatment will be evaluated using T cell receptor (TCR) repertoire analyses performed on extracted RNA.

Methodology is detailed in the accompanying Laboratory Manual and/or the Investigator's Brochure.

6.1.2.6 Relation Between HPV Status and Response to Therapy

- For both the CIN 3 and the VIN 3 cohorts, cervical HPV status including genotyping will be determined based on **cervical or vulvar swabs** obtained at baseline, during, and after treatment with the IRX-2 Regimen at time-points specified in the study protocol using a commercially available assay, Roche LINEAR ARRAY® HPV Genotyping Assay, or equivalent. Clearance of HPV from the cervix or vulva as determined by these assessments will be measured as a secondary endpoint of the study.
- The **tissue specimens** obtained during the study, including both the cervical and vulvar biopsies and excisions, will be evaluated for a) the presence or absence of different HPV genotypes; and b) expression of p16, using immunohistochemistry (IHC). The IHC will be performed using the paraffin embedded tissue blocks prepared by the clinical pathology lab.

Because some of the tissue biopsies may be of limited size, investigational use of specimens will always be secondary to the conventional histology needed for clinical management.

6.1.2.7 Activation, Clonality and Immunophenotypic Analyses of Peripheral Blood Lymphocytes

Peripheral blood mononuclear cells will be obtained by Ficol Hypaque centrifugation and cryopreservation of peripheral blood samples within 8 hours of collection. Peripheral blood lymphocytes will be evaluated by direct ex vivo ELISPOT analysis and TCR clonotypic analysis for evidence of induction of γ-interferon expressing T cells or T cell clones in response to HPV antigens. Baseline T cell reactivity and clonality will be compared to on-treatment and post-treatment levels.

Immunophenotypic flow cytometry will also be utilized to assess changes in naïve, memory, effector and regulatory T cell subsets.

The methodology to be used has been previously described by Dr. Martin Kast and his colleagues [Da Silva, 2016; Da Silva, 2015; van Poelgeest, 2016].

7 SURGICAL MANAGEMENT OF CIN 3 OR VIN 3

Surgical management of CIN 3 or VIN 3 is within the scope of practice for a gynecologist and so will only be summarized.

7.1 Initial Diagnostic Biopsies

The initial cervical or vulvar biopsies to establish the diagnosis of CIN 3 or VIN 3 will be done by the subject's referring clinician in the course of normal clinical care [Massad, 2013]. Upon enrollment in the trial of IRX-2 therapy, one of the clinical investigators will perform a baseline assessment of the subject, including a comprehensive colposcopy of the lower genital tract. Baseline photographs should be obtained. Biopsies will only be obtained if a previous biopsy has not been obtained or if slides/block from this are not available. This is comparable to what is normally done for a patient who is referred to a Colposcopy Clinic.

7.2 Therapeutic Excision of Dysplastic Lesion: Week 25

Following enrollment, each subject will receive two courses of therapy, the first administered during Week 1, and the second during Week 7. Six weeks later (Week 13), the dysplastic lesion is reevaluated by colposcopy, with photographic documentation, after which (week 25) it is surgically excised. In this trial the surgical excision will be performed but after a time period where immunotherapy will have had sufficient time to mediate a clinical effect (month 6 from start of treatment). Where progression at week 13 is suspected the surgical excision procedure would be moved earlier.

7.2.1 CIN 3 Cohort: Cervical Excision

The entire dysplastic lesion with a surrounding margin of transformation zone will be excised. For most subjects this can be accomplished with a loop electrosurgical excision procedure (LEEP); or for more superficial lesions, a large loop excision of the transformation zone (LLETZ). Subjects with more extensive lesions, including lesions extending into the endocervical canal, will require a deeper conization procedure (either a LEEP conization or a cone biopsy performed with conventional "scalpel" technique). If no lesions are visible at week 25, a LLETZ or cone biopsy via either LEEP or cold knife technique will still be performed for diagnostic purposes to rule out any occult dysplasia.

An endocervical curettage (ECC) will be done for all subjects at the time of the cervical excision, in keeping with standard of care, to rule out occult endocervical involvement with either dysplasia or cancer.

All decisions regarding the optimal type of excisional procedure to be performed (LLETZ, LEEP, scalpel conization); setting (office vs. operating room); and analgesia/anesthesia are to be made by the clinical investigator based on subject's needs and preferences.

7.2.2 VIN 3 Cohort: Wide Local Excision of Dysplastic Lesion

The size, location, and even geometry of a subject's dysplastic lesion or lesions will determine the optimal technique for surgical excision. Most VIN 3 lesions can be removed with a superficial wide local excision (WLE) as would be done with any cutaneous lesion. For larger lesions, an excision with adequate margins will require a superficial partial vulvectomy, and is usually done in the operating room for better subject comfort and safety. At week 25, if no VIN lesion is visible,

a punch biopsy of the original lesion site will be performed instead of WLE to confirm complete pathological response. Punch biopsies can be performed in a clinic setting.

7.2.3 Multifocal Lower Genital Tract Dysplasia, and/or Concurrent Genital Tract Condylomata

Multifocal lower genital tract dysplasia, with or without concurrent condylomata, is common, especially in younger women. While this protocol requires that the CIN 3 or VIN 3 lesion be surgically excised, the clinical investigator caring for the subject may treat other lesions in any manner consistent with standard of care, for example: concurrent CO2 laser ablation of vaginal dysplasia or vulvar condylomata at the time of excision of VIN 3.

Questions regarding permissible treatments in these circumstances should be referred to the PI.

8 STATISTICAL CONSIDERATIONS

8.1 Summary of Design (revised design – Amendment dated November 08, 2019)

This trial was originally designed as a two-cohort randomized placebo-controlled Phase 2 trial in women with squamous cervical intraepithelial neoplasia 3 (CIN 3) (Cohort 1) or squamous vulvar intraepithelial neoplasia 3 (VIN 3) (Cohort 2). Eligible patients would be randomized 2:1 to Regimen 1 or Regimen 2. In the experimental Regimen 1, patients receive IRX-2 injections (20 women in each cohort – 40 total) and in control Regimen 2 patients will receive placebo injections (10 women in each cohort – 20 total). Prior to randomization, patients were to be stratified by smoking history (current or recent smoking vs. not – see Section 5.2.2); after stratification, a permuted block design (with varying block sizes) was to be used to randomly assign the regimen. After randomization, patients were to receive two cycles of treatment followed by surgical resection of the CIN or VIN lesions.

Current, Revised Design

Based on slow accrual and in order to ensure completion in a timely fashion, the trial was redesigned to be a non-randomized, open-label trial. There will still be 2 cohorts of patients: (Cohort 1) those with squamous cervical intraepithelial neoplasia 3 (CIN3) and (Cohort 2) those with squamous vulvar intraepithelial neoplasia 3 (VIN3). This is still a Phase II/Screening trial.

The primary endpoint will be the complete or partial disappearance of all dysplasia (CR or PR) as determined by histologic examination of the Week 25 surgical specimen obtained at the time of surgical excision (LEEP, wide local excision, etc. – see Section 8.2.1); any patient who does not experience a CR or PR will be classified as being a "non-responder".

For this specific regimen to proceed to further clinical testing, we require a very strong signal - with at least 50% of subjects with CIN3, and with at least 40% of subjects with VIN3, experiencing the clear benefit of a CR or PR. A modest effect, while intriguing, would not be enough to justify further testing in otherwise healthy subjects.

It is known that some, relatively small, proportion of subjects with CIN 3 or VIN 3 will have no evidence of dysplasia in a subsequent excisional specimen or just a minimal disease remaining (CR and PR is defined in Section 6.1). Whether this represents complete removal of a small lesion with the initial diagnostic biopsy, an effect of post-biopsy inflammatory changes, or spontaneous resolution of the dysplastic lesion is impossible to ascertain for any one subject. In addition, all subjects in this trial will receive immuno-modulatory doses of cyclophosphamide, indomethacin, and zinc with multivitamins – any one of which may by itself affect immunologic clearance of the dysplastic lesion. Furthermore, repetitive injections of any substance into or below the dysplastic lesion may influence the resolution rate. Because subjects with very small lesions (which could be excised with the initial diagnostic biopsy) are excluded from this trial, a baseline rate of lesion regression or resolution is expected to be no more than 20% at the time of the "Week 25" excisional procedure for CIN [Trimble, 2005; Munk, 2007] and no more than 1.5% for VIN [van Seters, 2005].

After discussion with the team, it was determined that for the IRX-2 Regimen to be considered sufficiently promising for further study we would need to observe:

- in the population of subjects with CIN 3, the IRX-2 Regimen should result in a response rate that was clearly greater than 20% and not significantly less than 50%, and

- in the population of subjects with VIN 3, the IRX-2 injections should result in a response rate that was clearly greater than 5% and not significantly less than 40%.

Study Design. For each cohort, this trial will use a Simon two-stage design.

Cohort 1 (CIN3): with a false-positive error rate of 5% when the true objective response rate is 20% and a false-negative error rate of 10% when the true response rate is 50%. A total of 10 patients will be enrolled in the 1st stage. The first stage stopping rule is defined as observing only 2 or fewer responses among the first 10 patients. If 3 or more responses are observed, the trial will continue to a total of 22 patients. If 8 or more patients of the 22 experience a CR or PR then this will be evidence that the true response rate of IRX-2 Regimen is greater than 20% and not significantly less than 50%.

Cohort 2 (VIN3): with a false-positive error rate of 10% when the true objective response rate is 5% and a false-negative error rate of 10% when the true response rate is 40%. A total of 5 patients will be enrolled in the 1st stage. If there is no response observed among the first 5 patients, the enrollment will be stopped. If 1 or more responses are observed the trial will continue to a total of 10 patients. If 1 or more patients of the 10 experience a CR or PR then this will be evidence that the true response rate of IRX-2 Regimen is greater than 5% and not significantly less than 40%.

8.1.1 Toxicity Monitoring

Toxicity will be monitored on an ongoing basis during the conduct of the trial by the Study Team. To assist in this review of the proposed regimen, monitoring boundaries have been developed.

For these guidelines:

- ***Significant Local Toxicity*** will be defined as (a) any Grade 3 or greater AE or reaction (per CTCAE v4) related to study drug injections, at or near the site of injection, or (b) withdrawal from study due to intolerance of cervical or vulvar injection.
- ***Unacceptable Toxicity*** will be defined as any Grade 3 or greater AE, occurring during the observation period (from the time of the 1st injection until the Week 26 post resection evaluation) that cannot be clearly attributed to a non-IRX-2 treatment cause.
- Each of these toxicities will be monitored separately using the boundaries described below. CIN and VIN subjects who are treated with the IRX-2 regimen, will be combined to monitor toxicity. The toxicity monitoring boundaries are:
  - 2 or more of the 1st 10 subjects experience unacceptable toxicity
  - 3 or more of the 1st 15 subjects experience unacceptable toxicity
  - 4 or more of the 1st 20 subjects experience unacceptable toxicity

If the boundary is crossed for either definition of toxicity, this will serve as a flag to the Study Team to review the tolerability of the regimen and consider modifying some aspect of the regimen and amending the protocol.

8.1.1.1 Justification of the Monitoring Boundaries

The rules (2+/10 or 3+/15 or 4+/20) were selected to ensure a reasonable chance of crossing the boundary if the regimen produced either significant local toxicity or unacceptable toxicity (each monitored separately) in 20-25% of subjects. The table below summarizes the chance of crossing the boundary.

Chance of Unacceptable Toxicity	5%	10%	15%	20%	25%	30%
Probability of Crossing the Boundary (2+/10 or 3+/15 or 4+/20)	10%	31%	55%	74%	87%	94%

8.1.1.2 Toxicities related to indomethacin, cyclophosphamide, omeprazole and zinc-containing multivitamins

AEs including gastrointestinal symptoms such as heartburn or dyspepsia which could possibly be related to the indomethacin or hematologic changes such as anemia, neutropenia or thrombocytopenia which may be associated with cyclophosphamide will be monitored. Modifications to the study regimen for AEs related to these study drugs are described in Section 5.2.9.

8.2 Major Endpoints

8.2.1 Primary Endpoint: CR or PR in Surgical Specimen (≈week 25)

The primary endpoint is the response of CIN3 or VIN3 lesions to the IRX-2 Regimen as determined by histologic examination of the Week 25 surgical excision specimen. Complete response is defined as complete resolution of all dysplasia with no evidence of any dysplasia or cancer on the end of study pap smear, excisional procedure, ECC or biopsy. A partial response is defined as regression of CIN3 to CIN1 in cervical cases or VIN3 to VIN1 in vulvar cases based on the end of study excisional procedure or biopsy. In cervical cases where the internal margins of the cone are involved with CIN1, the pre-conization pap smear and post conization ECC must reveal no pathology more severe than CIN1.) (see Section 6.1).

8.2.2 Secondary Endpoints of Toxicity and Feasibility

Toxicity of the IRX-2 Regimen will be assessed by the incidence and severity of AEs and/or SAEs, as classified and graded according to the current version of the CTCAE v4, with one modification: attribution will be coded as definitely, possibly, and not related to treatment (per Section 9.1.2). All subjects will be assessed for AEs as detailed on the Study Schedules (Section 5.1) and evaluated for severity.

Feasibility for each patient will be determined if she (a) has received the 2 full cycles of treatment (specifically all 4 doses of IRX-2 in each cycle) and (b) can undergo surgical resection within the planned time window.

8.3 Secondary Endpoint of HPV status

An important endpoint in this trial will be the change in HPV status from before to after protocol treatment. All patients with CIN 3 on this trial will be "HPV positive" based on a commercial HPV genotyping assay which will be performed prior to treatment start; there is no such equivalent commercially available HPV genotyping assay for patients with VIN 3, and therefore patients with VIN 3 will be tested for HPV status after enrollment but before treatment using an assay in the USC Norris Immune Monitoring Core Laboratory, but this will not affect eligibility. Thus it is possible that 1 or 2 patients with VIN 3 will be "HPV negative" prior to start of treatment. Nonetheless, the HPV status, prior to start of protocol therapy, as well as at the time of surgical excision, will be known for all patients.

8.4 Secondary Endpoints-Other

A number of other exploratory secondary endpoints will be performed including immunophenotypic analysis of peripheral blood lymphocytes, frequency of serum antibodies and RNA expression profiling. These will be primarily descriptive end-points.

8.5 Accrual

Cervical dysplasia is more common than vulvar dysplasia, so study accrual will be different for each cohort. For the CIN 3 cohort, it is reasonable to expect enrollment of one subject per month, after an initial lag of two months when the study is first opened for recruitment. On average, there will be about a two-week interval between recruitment and Day 1 of treatment. With these considerations, all 22 CIN 3 subjects should be recruited by 22 months, and the surgical excision of the dysplastic lesion for the last recruited subject will occur around 28 months after opening the trial.

The VIN 3 cohort will probably enroll at a rate of one subject per quarter, so estimated completion of enrollment is at 30 months and performance of the last excisional procedure is at 36 ½ months. An active outreach program is planned to accelerate recruitment of the vulvar dysplasia subject to shorten this timeline as much as possible.

We expect approximately that 20% of patients may not complete therapy and/or surgical excision and therefore may not be fully evaluable. (Any such subjects will be contacted in keeping with clinic policies to ensure she receive appropriate care, irrespective of continued participation in the research study.) Thus about 8 additional patients may need to be enrolled adding approximately 3 months for completion of Cohort 1 and 6 months for completion Cohort 2.

The accrual times listed above may be shortened with the addition of any additional clinical sites.

8.6 Endpoint Analyses

All patients who received any amount of treatment will be accounted for; listings and summaries will include patients who receive at least one IRX-2 injection. For these patients, the following will be listed and summarized with standard descriptive methods: basic demographic and clinical baseline data, smoking status, number of cycles begun, number of injections received in each

cycle, reason off treatment, timing of surgical excision, amounts of cyclophosphamide, indomethacin, zinc-containing multivitamins, and omeprazole received, HPV levels, clinical response at each colposcopy, pathologic response, and toxicities (grade, type, cycle, and attribution) experienced.

8.6.1 Primary Endpoint: Complete Clearance or Major Regression (CR or PR) of Dysplasia Based on Surgical Excision Specimen

For the primary endpoint, the primary analysis will include all eligible patients who receive at least one cycle (4 injections) of IRX-2 and who undergo surgical excision. In an important secondary analysis, all eligible patients who receive at least one injection, will be included.

As stated above, the primary endpoint will be the complete or partial disappearance of all dysplasia (CR or PR) at the time of surgical excision (LEEP, wide local excision, etc.); any patient who does not experienced a CR or PR will be classified as having a "non-responder". An exact logistic regression will be used to test for an association between treatment and experiencing a CR/PR with history of smoking (currently or recently vs. not) as the single covariate in logistic model. Initially, the two cohorts of patients will be analyzed separately.

8.6.2 Secondary Endpoints: Toxicity and Feasibility

All patients who receive at least one injection of IRX-2 will be included in the analyses of toxicity and feasibility. All patients will be followed for adverse events as summarized in Section 6.1.

AEs will be recorded and classified by system organ class and preferred term coded in accordance with the version of CTCAE/MedDRA that is most recent at the start of the study - with one modification: attribution will be coded as definitely, possibly, and not related to treatment (per Section 9.1.2). Adverse event definitions and reporting requirements are detailed in Section 9.

Study participants will be summarized in terms of

- each type of AE at worst grade of severity
- any AE at worst grade of severity
- SAEs
- withdrawals (and reasons for them)
- dose modifications (and reasons for them)
- treatment interruptions (and reasons for them)
- abnormal laboratory findings that are considered clinically significant by the investigator
- any deaths occurring within 210 days (approx. 7 months) after Day 1 of treatment.

For reporting results, toxicities will be tabulated and reported according to regimen, cycle, toxicity type, grade, and attribution. The proportion of patients for whom the regimen was feasible (i.e. completed 2 cycles (received all 8 injections) and underwent surgical excision within the planned time period) will be calculated with associated (exact) 95% confidence intervals. For these analyses, both cohorts will be combined.

8.6.3 Additional Secondary Endpoints: Clinical CR or PR at Weeks 6, 13, and 25

The initial analysis will include all eligible patients who complete 1 cycles of therapy and undergo the 1st colposcopy at 6 weeks. The clinical response at weeks 6, 13, and 25 will be tabulated by Cohort and Regimen. The proportion of patients who achieve a CR's and PR's at each time point will be calculated with associated (exact) 95% confidence intervals. If numbers permit, changes over time will be summarized using models that accommodate repeated measures; the two Cohorts will be analyzed separately.

8.6.4 Additional Secondary Endpoints: HPV Status

HPV status will be assessed at baseline, at week 13 (at the time of planned colposcopy), at week 25 (at the time of surgical resection), and at week 41 (four months post excision or biopsy). All eligible patients who complete at least 1 cycle of treatment and have the HPV status assessed at week 13, 25, or 41 will be included in the initial analysis. The results at baseline and weeks 13, 25, and 41 will be tabulated by Cohort. Results will be reported as virus detected or not detected. Quantitative measurements of viral load will also be recorded.

8.6.5 Secondary/Exploratory Endpoints: Immunologic Studies

It is also planned that exploratory endpoints will be assessed in all subjects who undergo lesion excision, limited only by availability of histologic material (as explained in Section 6.1.2.4) if the quantity of biopsy material is small then priority is given to conventional diagnostic purposes over the performance of investigational studies). Analyses of some or all of the exploratory endpoints will be conducted by designated researchers at intervals depending on the availability of sufficient specimens. The Study Team may alter or discontinue some or all of these exploratory endpoints.

9 ADVERSE EVENTS, REPORTING REQUIREMENTS AND STOPPING RULES

9.1 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, omeprazole 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 the first day of treatment until the time of surgical excision of the dysplastic lesion (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 9.1.3.

Adverse events of Grade 3 or 4 will be reported to the Study Team and Medical Monitors, who will assess with the Site Principal Investigator whether to modify or discontinue study treatment.

Adverse events will be measured from Cycle 1 Day 1 until the week 25 surgical excision. Ongoing AE/SAEs will be reported and monitored until resolution. Evidence of toxicity after surgery will be assessed by tabulation of AEs felt by the investigators to be definitely or possibly related to the study regimen.

Serious adverse events will be reported, measured and monitored from the first day of treatment until the SAE resolves or the trial is terminated by the Study Team. All SAEs be tabulated regardless of relatedness to study drugs. Ongoing AEs at the time of surgery will be followed for 30 days post-surgery.

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

Pregnancy. While pregnancy is not an adverse event *per se*, any pregnancy or suspected pregnancy occurring during the clinical study or 1 year following the last dose of study medication should be reported to the Study Team and Medical Monitor immediately using the pregnancy report form. If the subject is still active in the study, the protocol drugs will be discontinued immediately and the subject removed from the trial. The Investigator will follow the subject until completion of the pregnancy and report pregnancy outcomes, including adverse maternal and neonatal outcomes. All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs.

9.1.1 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 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
- Required intervention to prevent permanent impairment/damage

9.1.2 Definition of Relationship to Study Drug

Association or relatedness to the study drug 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 drug administration.
  - Abates upon discontinuation of the drug.
  - 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 drug administration.
  - Abates upon discontinuation of the drug.
  - 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 drug administration.
  - Is explained by the subject's clinical state or by other modes of therapy administered to the subject.

9.1.3 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**

9.1.4 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

9.1.5 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

9.2 Notification of Serious or Unexpected Adverse Events

- For all sites- the sponsor/USC Principal Investigator, and USC DSMC and Brooklyn ImmunoTherapeutics must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.
    - USC Principal Investigator: Lynda Roman, MD

      Email: lroman@med.usc.edu

- Brooklyn ImmunoTherapeutics: SAE@linical.accelovance.com with cc to safety@brooklynitx.com

- The Institutional IRB must be notified of "any unanticipated problems involving risk to subjects or others" (UPR) in accordance with the Institutional policy.
- The following events meet the definition of UPR:

1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g., publication, safety monitoring report, updated safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
5. Any breach in confidentiality that may involve risk to the subject or others.
6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

- The USC NCCC Data and Safety Monitoring Committee (DSMC) must be notified within 24 hours of submission of such reportable event to the IRB. The patient ID and the study number as well as identifier of the SAE report should be submitted to the DSMC Coordinator via email (Grace.Kim@med.usc.edu) or Fax to the attention of the DSMC Coordinator at 323-865-0089.
- The FDA should be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.
    - USC: Study team to work with CISO QA to submit to the FDA using MedWatch3500A form in accordance within the FDA required timelines
    - Non-USC site: study team to notify USC PI, and USC DSMC Coordinator, within 24hrs of site becoming aware of the event by completing the MedWatch3500A form. USC will review and submit the applicable reports to the FDA and notify Brooklyn ImmunoTherapeutics of the submission.

10 ETHICAL AND REGULATORY CONSIDERATIONS

10.1 Protection of Human Subjects

This study has been designed and will be conducted in accordance with the principles outlined in the Declaration of Helsinki.

10.2 Informed Consent

Once a subject is referred for consideration in the study, that individual's history and current status will be completely evaluated, and treatment recommendations will then be discussed thoroughly with the subject. The investigator will discuss with the subject any other treatments that may be available and their relative value compared to the possible use of IRX-2 therapy. The risks and hazards of the procedures will be explained to the subject. The investigator shall seek consent only under circumstances that provide the individual sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the individual shall be in understandable language. No informed consent may include any exculpatory language through which the individual is made to waive or appear to waive any of their legal rights, or releases or appears to release the investigator, Brooklyn ImmunoTherapeutics, the institution, or its agents from liability for negligence. The subject, or legal guardian, must be able to comprehend the informed consent form and sign prior to enrollment. The subject will then receive a signed copy of the consent form.

10.3 Institutional Review Board or Independent Ethics Committee

The investigator must obtain approval for the study from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC). All changes to the protocol must be reviewed and approved prior to implementation, except where purely administrative or necessary to eliminate apparent immediate hazards to subjects. The investigator is responsible for obtaining annual IRB/IEC renewal through the duration of the study. Copies of the investigator's report and the IRB/IEC's continuance of approval must be submitted as directed.

11 STUDY ADMINISTRATION

11.1 Monitoring Plan

11.1.1 Pre-study Site Evaluation

The study monitor will visit each study center to confirm the site's qualifications for implementing this protocol. The protocol and study procedures will be reviewed with the investigator and study personnel. This visit may be combined with the study initiation visit or by completion of site qualification form.

11.1.2 Site Initiation

The study monitor will visit the site or perform the visit as a teleconference/WebEx to train personnel on all protocol elements, study procedures, and safety, and to confirm site readiness prior to enrollment. There may be visits to clinical, diagnostic laboratory, and pathology study participants or completion of site qualification form.

11.1.3 Interim Monitoring

The NCCC Data and Safety Monitoring Committee (DSMC) will monitor all NCCC initiated multi-center studies. If external sites have local policies and procedures that require them to perform internal auditing or data and safety monitoring of their patients enrolled to USC trials, the local auditing and/or monitoring schedule must be provided to the MCC team and USC PI. In addition, auditing and/or monitoring reports must be provided to the MCC team and USC PI within 2 weeks of issuance of the local report to the external site study team or local PI. Remote auditing will be conducted yearly or more frequently as needed, regardless of the external site's monitoring policies. The USC Audit Team, may elect to do an independent review if major issues are found on the local reports. The first two patients registered on a protocol will be audited. USC will notify external site study teams via an email reminder of an upcoming audit at least 2 weeks prior to a scheduled audit.

Auditing will be performed on multi-site protocols opened by the CISO-MCC. All reviews will be performed remotely, unless otherwise specified in a contractual agreement and agreed upon by both institutions. The MCC and external site study team will make available the required medical records, research files, regulatory documents and other documentation via a secure electronic method agreed upon by both parties. The Clinical Research Auditor will review these materials during the audit or monitoring review. Once the review for each subject selected is complete, a final report will be made available. Trends and other important findings will be reviewed during the standing trial and safety review teleconferences organized by the MCC. Specific findings for individual external sites will not be outlined during all-site teleconferences, but will rather be provided to the respective site during a dedicated teleconference with the site or via email, depending on the findings.

The CISO-MCC may require a "for cause" audit; this audit is required when significant findings or data indicate potential issues on the part of the site. The USC PI of the trial reserves the right to call for this audit with all sites.

If the audit reveals compliance issues, further follow up will include:

- A re-audit of the protocol in question by the MCC
- Suspension of an external site or the entire protocol to accrual or conduct at the discretion of the USC DSMC
- Recommendation of permanent closure of the protocol to the IRB; this is a rare occurrence and has to be done in consultation with USC PI and CISO leadership.

11.1.4 Study Close-out

At the study's conclusion, there will be a monitoring close-out visit at which time a final review and reconciliation of all study and regulatory documents and study drug will be performed.

11.1.5 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 may be made. Minor exceptions may be approved on a case-by-case basis. Any changes to the protocol will originate from the Investigator in the form of an amendment.

11.2 Compliance Statement

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, and the Food and Drug Administration Guidance for Industry: Computerized Systems Used in Clinical Trials.

11.3 Investigational Product Accountability

The principal investigator is responsible for investigational product accountability. The investigator or designee must maintain a current record of the receipt and disposition of investigational product, including dates, quantity, kit, lot, and subject numbers. The investigator or responsible designee will record the receipt, dispensation by subject number, kit number, lot number, and disposition of the product onto the drug accountability forms. The investigator will maintain a copy for the institutional records. Drug dispensing records must also be maintained.

In addition, a quality assurance release form and a shipping form (if applicable) will be included with each shipment stating lot number and quantity of product shipped. The investigator or responsible designee will sign the forms in the designated area, which certifies the receipt of supplies, and retain a copy for the institutional records. At sites that are dispensing drug, the investigator, or designee, will also maintain a dispensing record of IRX-2.

Used and unused drug vials will be destroyed by the responsible pharmacist at each clinical site after study monitor reconciliation procedures. Specific instructions will be provided in the Investigational Product Guidelines. Each site will be requested to use the procedures that are locally approved for destruction of biological products, and the details of such destruction will be captured on a form provided in the guidelines. These forms will be retained and available for inspection at the study site.

11.4 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 sponsor (USC), Brooklyn ImmunoTherapeutics or its designee, the FDA and other Regulatory Agencies. Elements should include, but are not limited to:

- Financial disclosure information to allow Brooklyn ImmunoTherapeutics to submit complete and accurate certification or disclosure statements required under Part 54 of Title 21 of the CFR. In addition, the investigators must provide to Brooklyn ImmunoTherapeutics a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study
- Study Files, containing the curriculum vitae of all investigators and his/her designees, FDA Form 1572, Financial Disclosure Forms, the protocol with all amendments, the IB, copies of all pre-study documentation, local lab certifications and lab normal ranges, and all correspondence to and from local Regulatory Authorities to Brooklyn ImmunoTherapeutics or its designee.
- Subject Files, containing the completed original CRFs, supporting source documentation and the signed informed consent form(s).
- Dispensing records of test agents.

The FDA requires that an investigator retain records for a period of at least 2 years from the date of FDA approval to market the product for this indication, or 2 years from the date that the application for approval is withdrawn by Brooklyn ImmunoTherapeutics. Please contact Brooklyn ImmunoTherapeutics or its designee prior to discarding any study-related files.

11.5 Study Closure/Study Termination

The investigational site or study may be terminated at any time for any reason, including but not limited to the following:

- Successful completion of the study
- Failure of the investigator to comply with the protocol or Good Clinical Practice/ International Conference on Harmonization guidelines
- Safety concerns
- Inadequate recruitment of subjects at a clinical site
- Decision to close the study for any reason

Disclosure of study termination will be made immediately to the IRB/IEC by the investigator.

11.6 Quality Assurance

Brooklyn ImmunoTherapeutics 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.

11.7 Confidentiality and Publication Policy

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

No written or oral reports or information about the trial, its progress, or results obtained for the duration of the trial will be provided by any investigator or anyone associated with this study, to anyone not involved in the trial other than to the Brooklyn ImmunoTherapeutics or its designees, or in confidence to the IRB/IEC, without the express, written permission of Brooklyn ImmunoTherapeutics.

Following the completion of the study, the data will be reported at a scientific meeting and by publication in a scientific journal at the discretion of the participating investigators, voting in proportion to the number of subjects each has entered on the study. Since this research effort is a multicenter investigation, each investigator acknowledges that participation in the protocol involves a commitment to present and/or publish the data from this study in a cooperative manner prior to doing so on an individual basis.

Any abstract or publication presenting results of this trial shall be given to Brooklyn ImmunoTherapeutics for review and approval at least 30 days prior to intended submission to a scientific journal for publication or to a scientific meeting for oral or poster presentation. Brooklyn ImmunoTherapeutics will not arbitrarily withhold or delay agreement to present the results of this study in appropriate scientific settings.

Brooklyn ImmunoTherapeutics endorses published guidelines for the publication and presentation of clinical study results. Brooklyn ImmunoTherapeutics and the investigators acknowledge their intent to resolve in good faith any differences of opinion regarding presentation or publication of study results.

12 STUDY MANAGEMENT

12.1 Conflict of Interest

All investigators will follow the University conflict of interest policy appropriate to their institution. Any USC investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must complete a "Statement of Outside Interests Related to Research" Form. The application is reviewed and approved by the Conflict of Interest Review Committee (CIRC) USC conflict of interest policy is available at http://ooc.usc.edu/conflict-interest-research

12.2 Institutional Review Board (IRB) Approval and Consent Process

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol and all study related documents used in the study (e.g. QOL questionnaire, pill diary, brochure, advertisement etc).

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing a dated IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person authorized to obtain the informed consent

12.3 Required Documentation (for multi-site studies)

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Investigation Support Office (CISO)

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- Protocol signature page with Investigator signature
- Form FDA 1572 appropriately filled out and signed with appropriate documentation (NOTE: this is required if {institution} holds the IND.
- A copy of the IRB approved consent form

- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract

12.4 Registration Procedures

Multi-Site Registration:

All participants in the multi-site trial are subject to central registration, which is used for tracking study accrual, checking eligibility, and monitoring adequate participation of women and minorities. Subject registration will be conducted through the coordinating center at the NCCC-CISO. External sites will identify eligible subjects and verify enrollment availability with the MCC prior to consenting patients. The external site is required to notify the MCC of a new signed informed consent within 48 business hours and note the basic consent information on the screening log. A copy of the consent will accompany the complete eligibility packet for verification. The MCC will enter the patient, demographic, and consent information in the applicable USC database. The MCC will assign a study patient sequence ID and communicate this to the external site.

The Coordinating Center Program Hours are 8 am to 4 pm, Monday through Friday, based on the PST zone. The MCC will be closed on official government holidays unless otherwise indicated. The contact number for the MCC is 323-865-3122. A copy of the registration sheet is located in the Appendix.

External sites will verify eligibility prior to submitting documents to the MCC for central registration. External sites must submit registration requests to the MCC at least one full business day prior to the planned treatment start date. Registration will require the external site to submit to the MCC all of the following:

- A completed registration form with patient demographics:
  - Zip code
  - Payor Source
  - Age
  - Sex
  - Race
  - Ethnicity
  - Initials
  - Date of Birth (DOB)
- A completed Eligibility Checklist signed by the investigator
- A copy of the most recently IRB-approved, patient signed informed consent form
- All required screening tests, within the time parameters specified by the protocol study calendar
- All other de-identified source documents needed to verify all points of eligibility

- Any On-Study forms for registration specified by protocol

These documents must be securely emailed to the MCC staff. With advance notice documents will also be accepted faxed to 323-865-0457. The MCC will verify completeness of documents and confirm eligibility. The MCC will enter the registration information in the USC OnCore® database. The MCC will then fax or securely email the completed Registration Form with the assigned study sequence ID to the external site as confirmation of patient registration.

An external site must maintain a log of all subjects who sign informed consents. The log must also document an explanation for exclusion due to screen failure. The MCC will provide sites with a Patient Tracking Log at the time of site activation. In the event of screen failure, external sites must submit the Screen Failure form to the MCC within one business day of determining screen failure.

Participating sites are required to retain, in a confidential manner, sufficient information on each subject so that the subject may be contacted should the need arise.

All documents, investigative reports, or information relating to the patient are strictly confidential. Any patient specific reports (i.e. Pathology reports, MRI reports, Operative reports, etc.) submitted to the CISO-MCC must have the patient's full name and social security number redacted (blacked out) and the assigned CISO-MCC patient ID number, protocol number, and site number written in. Patient initials only may be included or retained for cross verification of identification.

A registration verification letter will be emailed (preferred) or faxed to the registering site within one working day for patients registered to CISO-MCC multi-site trials. Treatment may not be initiated until the site receives this faxed or emailed verification.

USC Registration:

For patients enrolled at USC, the Research Coordinator must complete the protocol eligibility form to ensure that the patient is eligible. The PI will review the patient eligibility (with assistance from the Research Coordinator- who will assemble the required source documents, and do an initial review) prior to registering the patient on study.

The Research Coordinator or data manager will then register the patient into the Cancer Center database, Café, by accessing the Registration forms. Likewise, after the patient has completed the study, the Off Study forms in cafe will need to be completed, for Off Treatment and Off Study.

12.5 RECORDS AND DATA SUBMISSION

A. Confidentiality of Records

The original data collection forms will be kept in secure file cabinets, for USC patients forms will be kept in the Clinical Investigations Support Office (CISO). For additional clinical sites, original records may be stored locally in a secure fashion. Copies of data collection forms shall be sent to USC.

B. Patient Consent Form

At the time of registration, signed and dated copies of the patient Informed Consent with the Human Rights and the HIPAA authorization must be given to the patient. Institutional policy regarding distribution and location of original consent documents should be followed. When a study is opened at two or more institutions, a copy of the signed consent and HIPAA should be sent to USC CISO QA team as soon as possible, and not later than within 5 business days of obtaining consent. For patients consented at USC/LAC, institutional policy should be followed: a copy of ICF and HIPAA should be uploaded through True to USC CRO and to CISO QA Team. The original will be kept in the patient research chart maintained by the study assigned Data Manager.

C. Registration Eligibility Worksheet

At the time of registration, the completed Eligibility Worksheet will be submitted to the QA Monitor at CISO for review of eligibility compliance.

D. Data Collection Forms and Submission Schedule

If a treatment trial, protocol data will be entered into eCRFs in Café.

Within two weeks of registration, the data manager will complete the initial set of On Study forms and baseline Toxicities.

Within two weeks of completion of each course of treatment, the data manager must complete the Course Assessment, Toxicities, and if appropriate Response data.

After Off Treatment, within two weeks of each follow up, complete the Follow Up forms.

12.6 Data Management and Monitoring/Auditing

12.6.1 Active Monitoring Program Details

a. Adherence to Protocol/Per Patient: It is the responsibility of the USC Principal Investigator (PI) to ensure that patient recruitment and enrollment, treatment, follow-up for toxicities and response, and documentation and reporting at USC are all performed as specified in the protocol. When a study is opened at two or more institutions, the PI at each institution will assume the responsibilities for the day-to-day monitoring of the trial, as described below.

b. Day-to-Day Monitoring – Eligibility: At USC, the Study Coordinator will assist the Investigator in reviewing eligibility and will assemble the required source documents, and do a final review by completing an Eligibility Registration Worksheet. When a study is opened at two or more institutions, the PI at each institution will review the patient eligibility in accordance with that institution's policy. For all institutions, the Eligibility Registration Worksheet with a copy of Informed Consent and supporting source documents will be submitted to CISO QA via email or Fax for verification prior to registering the patient on study.

c. Day-to-Day Monitoring – Informed Consent: Prior to registering the patient on study, the Study Coordinator will review the informed consent, to ensure that the patient has signed and dated the most current IRB-approved form, and that the form has been signed and dated by the person obtaining the consent as well as appropriate witnesses. A copy of the ICF will also be provided to CISO QA for review. CISO SOP 3.3 will be followed.

d. Day-to-Day Monitoring – Treatment: The PI and co-investigators are responsible for ensuring that treatment is given per protocol. The Study Coordinator will review the treatment orders with the treating investigator. Regardless of who the treating physician is, there will be only one responsible Study Coordinator for each study at each of the hospitals affiliated with the USC Norris Cancer Center. The treating investigator will review the status of each patient on-study, with the Study Coordinator and treating physicians, on an on-going basis. When a study is opened at two or more institutions, CISO QA will periodically audit medical records for the subjects on study at other institutions to ensure compliance and adherence to the protocol.

e. Data Management – Patient Charts: When a study is opened at two or more institutions, the policy in place at each institution will be followed for maintaining medical and research related records. Such policies will be provided to the CISO QA prior to enrolling 1st patient. At USC, All written source documents not associated with the study research are maintained in the patient chart, which is stored in the Department of Medical Records at the appropriate hospital. At the Norris Hospital, the official medical record is the Electronic Patient File (EPF). Radiographical images are stored in the Department of Radiology and in an electronic system called Synapse. At Los Angeles County General Hospital the official medical record is called ORCHID. These are the permanent, official documents for each patient on-study. A copy of the signed informed consent, physician's notes, orders, test results and pathology notes are maintained in the patients' hospital charts. It is the responsibility of the research staff to ensure that the patient chart contains the required documents and work closely with treating investigators to ensure all protocol-related assessments are carefully documented.

f. Data Management – Research Charts: When a study is opened at two or more institutions, the policy in place at each institution will be followed for maintaining medical and research related records. Such policies will be provided to the CISO QA prior to enrolling 1st patient. At USC, to facilitate adherence to

the protocol schedule and data management, research charts are created to collect copies of the relevant notes, orders and results, that are in the Patient Chart. In addition, all source documents related to the research, such as original informed consent forms, HIPAA Forms, AE assessment worksheets, disease response worksheets and NTFs are maintained in the Research Charts. Protocol calendars, worksheets, and checklists, are also kept in the research chart. These are maintained in the Clinical Investigation Support Office until the study is completed and the results are published and no further need is anticipated. These are then stored off-site. It is the responsibility of the Data Manager to ensure that the research chart contains all the required documents.

g. Data Management – Case Report Forms: It is the responsibility of the Data Manager to complete the required case report forms. For in-house trials, case report forms are developed for each trial; these are used to finalize the data entry screens in the Cancer Center clinical trials database. It is the responsibility of the PI to review the Off-Study Summary form which summarizes pertinent toxicity, response and adherence information, once the patient has completed treatment.

12.6.2 Quality Assurance Monitoring Committee (QAMC) Oversight

The Quality Assurance and Monitoring Committee (QAMC) of the NCCC has the responsibility for study auditing and monitoring for protocol compliance, data accuracy, performance of audits and monitoring of accrual. QAMC procedures are detailed in the NCCC Data Safety and Monitoring Plan available on CISO Website.

12.6.2.1 QAMC Annual Patient Audits

The QAMC is responsible for conducting audits and providing the initial review of the audits, for all open institutional (i.e. USC initiated), CCCP-sponsored trials, and any trials identified by the CIC. These trials are audited by the QAMC once a year. Faculty and staff at the Cancer Center involved in clinical research – but not directly involved in the research under evaluation – are asked to serve as auditors. Twenty percent of patients accrued during the past 12 months – and a minimum of 2 patients – are selected at random; however, additional patients may be selected for audit if there is some indication that there might have been a problem or unusual circumstance (possibly related to compliance, toxicity, response or some indication of an irregularity). The audit involves a review of the research chart, hospital medical record (i.e., source documentation) and evaluates the following: documentation of eligibility (including failure to obtain appropriate informed consent) and baseline status of the patient; documentation of adherence to protocol-specified treatment and follow-up; evaluation of toxicity; and evaluation of response or other outcome. In addition, for investigative agents, a drug audit is also performed for these patients by the Research Pharmacist. In addition, for Institutional,

Investigator Initiated Trials, Data in the CAFÉ database are compared to the information in the medical record.

12.6.2.2 QAMC Annual Protocol Review

All open trials are reviewed at least once a year by the QAMC (or more often if stipulated by the CIC). This annual review includes the following: evaluation of the current accrual relative to the planned total accrual; examination of gender and minority accrual; examination of all reported violations; review of past audits and correspondence with the PI; review of results of current audit (by an outside agency or by the NCCC QAMC); review of previous correspondence between the PI and the QAMC/DSMC. The QAMC review process is detailed in USC NCCC DSM Plan available on the CISO website.

12.6.3 Data and Safety Monitoring Committee (DSMC) Oversight

The Data and Safety Monitoring Committee (DSMC) is an independent body responsible for the safety of study subjects through the review of new protocols to ensure an adequate adverse event assessment/reporting plan, study stopping rules and through the real-time and periodic monitoring of severe adverse events (SAEs) or those AEs that require expedited reporting. The DSMC performs quarterly and annual safety reviews as well as interim efficacy/futility analyses on institutional trials. DSMC procedures are detailed in USC NCCC DSM Plan available on the CISO website.

12.6.4 Phase I Committee Oversight (for Phase I or I/II IIT only)

The USC Norris Comprehensive Cancer Center Phase I DLT committee reviews all open institutional phase I studies at regularly scheduled intervals. The committee reviews the adverse events, serious adverse events, and treatment administration for each patient during the DLT observation period as specified per protocol. The committee will determine whether a patient is evaluable for DLT and whether an AE meets the DLT definition or not. Decisions regarding dose escalation, de-escalation and cohort expansion are made by the committee in coordination with the PI.

12.7 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

12.7.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

12.7.2 Non-Emergency departures from protocol

A protocol deviation is any variance from an IRB approved protocol.

If the deviation meets all of the following criteria, it is considered a minor protocol deviation that:

- Is generally noted or recognized only after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

If the deviation meets any of the following criteria, it is considered a protocol violation:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious noncompliance with federal regulations, State laws, or University policies.

Protocol Deviations: personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies.

Protocol Violations: All protocol violations will be entered in the clinical trial database by the Research Coordinator. In addition, Research Coordinator and Investigator should report all protocol violations within one (1) week of the knowledge of the event using iStar.

12.7.3 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB as well as to all the sponsoring agencies (FDA, NCI, etc.) for review and for approval prior to implementation. It is the responsibility of the study PI to ensure that the appropriate agencies have been informed of the proposed amendments and that these have been reviewed and approved.

12.8 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Brooklyn ImmunoTherapeutics-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

12.9 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

13 REFERENCES

Committee on Gynecologic Practice of American College of Obstetricians and Gynecologist. ACOG Committee Opinion no. 509: management of vulvar intraepithelial neoplasia. Obstet Gynecol 2011;118:1192.

Committee on Practice Bulletins – Gynecology. ACOG Practice Bulletin Number 131: Screening for cervical cancer. Obstet Gynecol 2012; 120:1222

Alani RM, Munger K. Human papillomaviruses and associated malignancies. J Clin Oncol 1998;16:330.

Albrechtsen S, Rasmussen S, Thoresen S, et al. Pregnancy outcome in women before and after cervical conisation: population based cohort study. BMJ 2008;337:a1343.

Aldara Prescribing Information, 2010;
http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020723s022lbl.pdf

Arbyn M, Kyrgiou M, Simeons C, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ 2008;337:a1284.

Bachaud JM, Cohen-Jonathan E, Alzieu C, et al. Combined postoperative radiotherapy and weekly cisplatin infusion for locally advanced head and neck carcinoma: final report of a randomized trial. Int J Radiat Oncol Biol Phys. 1996;36:999-1004.

Baldauf JJ, Dreyfus M, Ritter J, Meyer P, Philippe E. Risk of cervical stenosis after large loop excision or laser conization. Obstet Gynecol 1996;88:933

Baldwin PJ, van der Burg SH, Boswell CM, et al. Vaccinia-expressed human papillomavirus 16 and 18 E6 and E7 as a therapeutic vaccination for vulval and vaginal intraepithelial neoplasia. Clin Cancer Res 2003;9:5205-13.

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.

Bernier J. Cetuximab in the treatment of head and neck cancer. Expert Rev Anticancer Ther 2006;11:1539-52.

Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without conconmitant chemotherapy for locally advanced head and neck cancer. NEJM 2004;19:1945-52.

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

Bosch X, Harper D. Prevention strategies of cervical cancer in the HPV vaccine era. Gynecol Oncol 2006;103:21.

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.

Bruinsma FJ, Quinn MA. The risk of preterm birth following treatment for precancerous changes in the cervix: a systematic review and meta-analysis. BJOG 2011;118:1031-41.

Centers for Disease Control and Prevention (CDC). Human Papillomavirus Vaccination Coverage Among Adolescent Girls, 2007–2012, and Postlicensure Vaccine Safety Monitoring, 2006–2013—United States. MMWR Recomm Rep. 2013;62:591

Chung CH, Zhang Q, Kong CS, et al. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. J Clin Oncol. 2014;32:3930-8.

Cooper JS, Pajak TF, Forastiere AA, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. NEJM 2004;19:1937-44.

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.

Darragh TM, Colgan TJ, Thomas Cox J, et al. The Lower Anogenital Squamous Terminology Standardization prject for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol 2013;32:76.

Da Silva DM, Woodham AW, Naylor PH, Egan JE, Berinstein NL, Kast, WM. Immunostimulatory activity of the cytokine-based biologic, IRX-2, on human papillomavirus-exposed Langerhans cells. J Interferon Cytokine Res. 2016;36(5):291-301. doi:10.1089/jir.2015.0115

Da Silva DM, Woodham AW, Skeate JG, et al. Langerhans cells from women with cervical precancerous lesions become functionally responsive against human papillomavirus after activation with stabilized Poly-I:C. 2015;161(2):197-208.

Davidson EJ, Boswell CM, Sehr P, et al. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. Cancer Res 2003;63:6032-41.

Deacon JM, Evans CD, Yule R, et al. Sexual behavior and smoking as determinants of cervical HPV infection and of CIN 3 among those infected: a case-control study nested within the Manchester cohort. Br J Cancer 2000;83:1565.

Dueñas-Gonzalez A, Verastegui E, Lopez-Graniel C, Gonzalez A, Mota A, Barrera-Franco JL, et al. A pilot study of perilymphatic leukocyte cytokine mixture (IRX-2) as neoadjuvant treatment for early stage cervical carcinoma. International Immunopharmacol 2002;2:1007-1016.

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.

Fahey LM, Raff AB, Da Silva DM, Kast WM. Reversal of human papillomavirus-specific T cell immune suppression through TLR agonist treatment of Langerhans cells exposed to human papillomavirus type 16. J Immunol. 2009;182:2919-28.

Fausch S, Fahey LM, Da Silva DM, Kast WM. Human papillomavirus can escape immune recognition through Langerhans cell phosphoinositide 3-kinase activation. J Immunol 2005;174:7172-7178.

FDA News Release, December 10, 2014. FDA approves Gardasil 9 for prevention of certain cancers caused by five additional types of HPV. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm426485.htm

Forman D, de Martel C, Lacey CJ. Global burden of human papillomavirus and related diseases. Vaccine 2012;30:Supplement 5.

Fox H, Wells M. Recent advances in pathology of the vulva. Histopathology 2003;42:209.

Frazer IH. The role of vaccines in the control of STDs: HPV vaccines. Genitourin Med 1996;72:398-403.

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.

Friedrich ER Jr, Wilkinson EJ, Fu YS. Carcinoma in situ of the vulva: a continuing challenge. Am J Obstet Gynecol 1980;136:830.

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

Giuliano AG, Sedjo RL, Roe, DJ. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes and Control. 2002;13:839–846.

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.

Jakobsson M, Gissler M, Paavonen J, Tapper AM. Long-term mortality in women treated for cervical intraepithelial neoplasia. BJOG 2009a;116:838-44

Jakobsson M, Gissler M, Paavonen J, Tapper AM. Loop electrosurgical excision procedure and the risk for preterm birth. Obstet Gynecol 2009b;114:504-10

Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, et al. Five-year risk of cervical cancer and CIN3 for HPV-positive and HPV-negative high-grade Pap results. J Low Genit Tract Dis 2013;17:S50-S55

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

Kenter GG, Welters MJP, Valentijn ARPM, Lowik MJG, Berends-van der Meer DMA, Vloon APG, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med 009;361:1838-47.

Khalid S, Dimitriou E, Conroy R, Paraskevaidis E, Kyrgiou M, Harrity C, et al. The thickness and volume of LLETZ specimens can predict the relative risk of pregnancy related morbidity. BJOG 2012;119;685-91.

Kyrgiou M, Koliopoulos G, Martin-Hirsch P, et al. Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systemic review and meta-analysis. Lancet 2006;367:489.

Kyrgiou M, Mitra A, Arbyn M, et al. Fertility and early pregnancy outcomes after treatment for cervical intraepithelial neoplasia: systematic review and meta-analysis. BMJ 2014;349:g6192.

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.

Lee KR, Darragh TM, Joste NE, Krane JF, Sherman ME, Hurley LB, et al. Atypical glandular cells of undetermined significance (AGUS): interobserver reproducibility in cervical smears and corresponding thin-layer preparations. Am J Clin Pathol 2002;117:96-102

Lerner D, Parsons SK, Justicia-Linde F, Chelmow D, Chang H, Rogers WH, et al. The impact of pre-cancerous cervical lesions on functioning at work and work productivity. J Occup Environ Med. 2010;52:926-33.

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.

Mahto M, Nathan M, O'Mahony C. More than a decade on: review of the use of imiquimod in lower anogenital intraepithelial neoplasia. Int J STD AIDS 2010;21:8.

Martin PJ, Davenport-Ennis N, Petrelli NJ, et al. Responsibility for costs associated with clinical trials. J Clin Oncol. 2014;32:3357-9.

Martin-Hirsch PPL, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. Cochrane Database Syst Rev 2013;12: CD001318. doi: 10.1002/14651858.CD001318.pub3

Massad LS, Einstein, MH, Huh WK, et al. 2012 Updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1.

Melnikow J, McGahan C, Sawaya GF, et al. Cervical intraepithelial neoplasia outcomes after treatment long-term follow-up from the British Columbia Cohort Study. J Natl Cancer Inst 2009;101:721.

Montz FJ. Management of high-grade cervical intraepithelial neoplasia and low-grade squamous intraepithelial lesion and potential complications. Clin Obstet Gynecol 2000; 43:394.

Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA. 2001;285:2995-3002.

Moscicki AB, Ma Y, Wibbelsman C, et al. Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women. Obstet Gynecol 2010;116:1373.

Munk AC, Kruse AJ, van Diermen B, et al. Cervical intraepithelial neoplasia grade 3 lesions can regress. APMIS 2007;115(12):1409-14.

Muntz HG. Cervical cancer screening and management of the abnormal Papanicolaou smear. In: Current Care of Women: Diagnosis & Treatment. 2nd Edition. Lemcke DP, Pattison J, Marshall LA, Cowley DS (eds). McGraw-Hill/Appleton & Lange, New York, NY, 2004, pp. 520-531.

Muntz HG. Can cervical adenocarcinoma in situ be safely managed by conization alone? Gynecol Oncol 1996;61:301-303

Muntz HG, Bell DA, Lage JM, Goff BA, Feldman S, Rice LW. Adenocarcinoma in-situ of the uterine cervix. Obstet Gynecol 1992;80:935-939

National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, June 14, 2010.

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.

Noehr B, Jensen A, Frederiksen K, et al. Loop electrosurgical excision of the cervix and subsequent risk for spontaneous preterm delivery: a population-based study of singleton deliveries during a 9-year period. Am J Obstet Gynecol 2009;201:33.e1.

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.

Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.

Paraskevaidis E, Arbyn M, Sotiriadis A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G, et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. Cancer Treat Rev 2004;30:205-11.

Rebolj M, Helmerhorst T, Habbema D, et al. Risk of cervical cancer after completed post-treatment follow-up of cervical intraepithelial neoplasia: population based cohort study. BMJ 2012;345:e6855.

Reyes MC, Cooper K. An update on vulvar intraepithelial neoplasia: terminology and a practical approach to diagnosis. J Clin Pathol 2014;67:290.

Rutledge F, Sinclair M. Treatment of intraepithelial carcinoma of the vulva by skin excision and graft. Am J Obstet Gynecol 1968;102:807.

Sadler L, Saftlas A, Wang W, Exeter M, Whittaker J, McCowan L. Treatment for cervical intraepithelial neoplasia and risk of preterm delivery. JAMA 2004;29:2100-6.

Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993;85:958.

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.

Sideri M, Jones RW, Wilkinson EJ, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. J Reprod Med 2005;50:807.

Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. CA Cancer J Clin 2015;65:5-29

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

Smyth LJ, van Poelgeest MI, Davidson EJ, et al. Immunological responses in women with human papillomavirus type 16 (HPV-16)-associated anogenital intraepithelial neoplasia induced by heterologous prime-boost HPV-16 oncogene vaccination. Clin Cancer Res 2004;10:2954-61.

Solomon D, Davey D, Kuman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2114.

Stanley MA, Pett MR, Coleman N. HPV: from infection to cancer. Biochemical Society Transactions 2007;35,part 6:1456-60.

Svare JA, Andersen LF, Langhoff-Roos J, et al. The relationship between prior cervical conization, cervical microbial colonization and preterm premature rupture of the membranes. Eur J Obstet Gynecol Reprod Biol. 1992;47:41.

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.

Tota JE, Chevarie-Davis M, Lyndsay A. Richardson LA. Epidemiology and burden of HPV infection and related diseases: Implications for prevention strategies. Preventive Medicine 2011;53:S12–S21.

Trimble CL, Piantadose S, Gravitt P, et al. Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. Clin Cancer Res. 2005;11(13):4717-23.

van Poelgeest MI, Welters MJ, Vermeij R, et al. Vaccination against oncoproteins of HPV16 for noninvasive vulvar/vaginal lesions: lesion clearance is related to the strength of the T-cell response. Clin Cancer Res. 2016;22(10):2342-50.

van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol 2005; 97:645

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.

Widgren K, Simonsen J, Valentiner-Branth P, Mølbak K. Uptake of the human papillomavirus-vaccination within the free-of-charge childhood vaccination programme in Denmark. Vaccine. 2011 Dec;29(52):9663-7. Epub 2011 Oct 18

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.

Woodham AW, Raff AB, Da Silva DM, Kast WM. Molecular analysis of human papillomavirus virus-like particle activated Langerhans cells in vitro. Methods Mol Biol. 2015;1249:135-49.

Workowski KA, Bolan GA. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep 2015;64:1.

Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: results from a large academic women's hospital laboratory employing sensitive screening methods. Gynecol Oncol 2009;114:383-9

Appendix 1: Cervical Intraepithelial Neoplasia Nomenclature

Descriptive Terminology		**Mild Dysplasia**	**Moderate Dysplasia**	**Severe Dysplasia**	**Carcinoma in situ**
Bethesda Classification System[a]	Cytology	LSIL	HSIL		
	Histology	CIN 1	CIN 2	CIN 3	
LAST System[b]	Cytology	LSIL	HSIL		
	Histology	LSIL	Perform p16 staining: negative is LSIL positive is HSIL	HSIL	

a. Solomon, 2002
b. Darragh, 2013

Appendix 2: Vulvar Intraepithelial Neoplasia Nomenclature

Descriptive Terminology		**Mild Dysplasia**	**Moderate Dysplasia**	**Severe Dysplasia**	**Carcinoma in situ**
VIN Terminology (Derived from Bethesda System)[a]		VIN 1	VIN 2	VIN 3	
ISSVD System[b]	HPV Associated	Condyloma Acuminatum	VIN, usual type		
	Antecedent Vulvar Dystrophy	N/A (e.g., Lichen Sclerosus)	VIN, differentiated type*		
LAST System[c]		Condyloma Acuminatum	HSIL		

a. Reyes, 2014
b. Sideri, 2005
c. Darragh, 2013

*Subjects with the non-HPV associated, or differentiated type of VIN, are excluded from enrollment in this trial.

Appendix 3: ECOG Performance Status

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

Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.

Appendix 4: Optimal Schedule for USC Investigators, Based on GYN Clinic Schedule

		IV Cytoxan given on Day 1 Friday of each cycle. GYN Clinic days on Monday through Thursday, Days 4-7 each cycle, for the 4 daily IRX-2 sub-lesional injections						
		Fri	Sat	Sun	Mon	Tues	Wed	Thurs
Week		------ Cycle 1 ------						
1		1	2	3	4	5	6	7
2		8	9	10	11	12	13	14
3		15	16	17	18	19	20	21
4		22	23	24	25	26	27	28
5		29	30	31	32	33	34	35
6		36	37	38	39	40	41	42
Week		------ Cycle 2 ------						
7	**(1)**	43 (1)	44 (2)	45 (3)	46 (4)	47 (5)	48 (6)	49 (7)
8	**(2)**	50 (8)	51 (9)	52 (10)	53 (11)	53 (12)	55 (13)	56 (14)
9	**(3)**	57 (15)	58 (16)	59 (17)	60 (18)	61 (19)	62(20)	63 (21)
10	**(4)**	64 (22)	65 (23)	66 (24)	67 (25)	68 (26)	69 (27)	70 (28)
11	**(5)**	71 (29)	72 (30)	73 (31)	74 (32)	75 (33)	76 (34)	77 (35)
12	**(6)**	78 (36)	79 (37)	80 (38)	81 (39)	82 (40)	83 (41)	84 (42)
Week		Week 13 Safety Assessment (Day 91)						
13		85	86	87	88	89	90	91
21		Pre-surgical assessment Week 21 (Day 147 ±14 days)						
25		Surgical Resection Week 25 (Day 175 ±7 days)						
26+		Post-Op Check Week 26+ (1 to 8 weeks after surgery)						

APPENDIX 5

REGISTRATION REQUEST FORM

PROTOCOL NUMBER:_____

Patient Initials (First-Middle-Last):	
Address (Zip Code):	Birth Date:
	Sex:
Race (Please check all that apply): ☐ American Indian or Alaska Native ☐ Black or African American ☐ White ☐ Asian ☐ Native Hawaiian or Pacific Islander ☐ Other	
Ethnicity (Please check): ☐ Hispanic ☐ Non-Hispanic ☐ Other	
U.S. Resident?: ☐ Yes ☐ No If No, enter Country of Residence:	
Method of Payment (Please check): ☐ Private Insurance; ☐ Medicare; ☐ Medicare & Private Insurance; ☐ Medicaid; ☐ Medicaid & Medicare; ☐ Military or Veterans Administration Sponsored; ☐ Self Pay (No Insurance); ☐ No Means of Payment (No Insurance); ☐ Unknown	
Date IC Signed _____/_____/_____ **Time** _____ **am/pm**	**Date HIPAA Signed** _____/_____/_____
Date of Request Form Submitted:	Site Name:
Site Telephone #:	Site Principal Investigator Name:

By signing below, the investigator attests to the review of the source documents for the protocol eligibility requirements.

_____

Eligibility reviewed by: Investigator Name

_____ _____

Investigator Signature **Date**

Utilize the signed form as the cover sheet for the de-identified source doc submission when requesting MCC eligibility verification and registration

Appendix 6: Pill Diary
Protocol # 5GYN-16-2
Give Diary with Start of Each New Cycle

Patient's Name: ________________ ________________ CISO Study ID: ________ Medical Record #: ______________
Study Drug Name: ______________________ Daily Dosage Prescribed: ____
Frequency per day: _____________ Number of oral drugs (pills, capsules) dispensed: ______________________________
Cycle#: _____________ Date to Begin: _____/_____/_____ Date Form Given to Patient: _____/_____/_____

DRUG administration instructions: ____
____
____

Patient: *PLEASE RECORD WHEN (DATE & TIME) YOU TOOK YOUR MEDICATION IN THE CALENDAR BELOW*

Month_________________ **Year** _____________

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______
☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______
☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______
☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______
☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______	☐ A.M.: ______ P.M.: ______

MISSED DOSES: If a dose was missed, please record the reason why it was ***not taken***, along with amount, time and date (for example forgot to take 1 pill at 8 P.M. on 1/22/02.):____
____
____

OTHER COMMENTS: ____
____

PLEASE RETURN ALL UNUSED MEDICATION TO YOUR RESEARCH NURSE AT THE NEXT VISIT.

Patient Signature: ________________________ **Date Diary Returned**: ___/___/___

__________ - __________ = __________ ÷ __________ **x 100 =** __________ **% taken**
(# Dispensed) (# Remaining) (# Taken) (# Dispensed)

Revised 5.31.2011 **Initial of Research Nurse** ___ ___