TITLE: A Phase I/IIb Study of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC in Combination with INCB024360 for Patients in Remission with Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma Whose Tumors Express NY-ESO-1 or LAGE-1 Antigen

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Confidentiality Statement

SYNOPSIS

Title / Phase	A Phase I/IIb Study of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC in Combination with INCB024360 for Patients in Remission with Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma Whose Tumors Express NY-ESO-1 or LAGE-1 Antigen
Roswell Park Study Number	I 248613
Roswell Park Clinical Investigator	Emese Zsiros, MD
Study Drugs	DEC205mAb-NY-ESO-1 fusion protein given with adjuvant poly-ICLC INCB024360 Exploratory Cohort at Roswell Park ONLY: Sirolimus
Objectives	**Phase I Primary Objectives:** • To determine the safety of fixed doses of DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC given as a vaccine in combination with INCB024360. • To evaluate toxicity as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. **Phase IIb Primary Objective:** • To determine the progression free survival (PFS) (primary endpoint) using irRC criteria. **Phase I and Phase IIb Secondary Objectives** • To determine the effectiveness of INCB024360 on enhancing vaccine efficacy by assessing NY-ESO-1 specific cellular and humoral immunity. • Exploratory Cohort at Roswell Park ONLY: To determine the effectiveness of Sirolimus on enhancing vaccine efficacy by assessing NY-ESO-1 specific cellular and humoral immunity. o Peripheral blood NY-ESO-1 specific CD8+ and CD4+ T cells. o Peripheral blood NY-ESO-1 specific antibodies. o Peripheral blood frequency of CD4+CD25+FOXP3+ regulatory T cells. o Pharmacokinetics of INCB024360 in relation to T cell frequency and function in correlation with PFS.
Study Design	This is an open-label, Phase I/IIb study of the IDO inhibitor, INCB024360, in combination with DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC in patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in remission. For the Phase I component of the study, eligible patients will receive a fixed intracutaneous dose of DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with subcutaneous adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles; and Cohort 1 patient enrollment will begin INCB024360 at a fixed daily dose of 300 mg BID orally (Cohort 1) for a total of 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -1. Cohort -1 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 100 mg BID (Cohort -1) starting on Day 1 for 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -2. Cohort -2 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant

	poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 50 mg BID (Cohort -1) starting on Day 1 for 7 cycles. Phase IIb: The Phase IIb component of this trial will consist of a randomized parallel design of n=25+25=50 patients =50 patients with treatment arms: (i) DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC alone and (ii) INCB024360 in combination with the DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC. Stratification will be on clinical site (Roswell Park, U. of Pittsburgh). The primary endpoint will be progression free survival. Baseline is defined as the time following the treatment free interval, which can span 0 - 2 months, as described in Iasonos et al.[1] The primary analysis will be carried forth using a Cox proportional hazards model with factors corresponding to treatment and a continuous covariate adjustment for the length of the treatment free interval and a stratification factor for clinical site. Exploratory Cohort Roswell Park site ONLY: An additional exploratory cohort (N=6) receiving 4 cycles of sirolimus in combination with DEC205mAb-NY-ESO-01 fusion protein (CDX-1401) with adjuvant poly-ICLC will be added to supplement the secondary objectives, which will aid in planning the next phase of the investigation. This will not affect the randomized component of the study as enrollment to the randomized Phase IIb at Roswell Park only will be suspended temporarily until the 6 patients are enrolled.
Accrual Target / Duration	**Phase I:** 3 – 12 patients **Phase IIb:** 50 patients **Exploratory Cohort**: 6 patients The duration of patient enrollment is expected to be 8 years. Patients in Phase I and Phase IIb are expected to participate in the study for approximately 28 weeks of active therapy. Patients in the exploratory cohort are expected to participate in the study for approximately 20 weeks of active therapy.
Study Procedures and Observations	Patients will have regularly scheduled study visits at the clinical site including physical exam, hematology, chemistry, and performance status-refer to Table 3 and Table 4 schedule of procedures and observations for specific time points.. **Disease Assessment:** Baseline, Cycle 4 Day 1, treatment discontinuation, and 3 and 6 months after last study drug dose. **Adverse Events:** From first dose of study drug until 30 days (± 3 days) after the last dose of study drug.

Statistical Analysis	• **Phase I:** The following **decision rules** will be followed: (i) If 0/3 dose limiting toxicities (DLTs) are observed proceed to enroll 3 more patients (ii) If 1/3 DLTs are observed enroll 3 more patients at the same treatment regimen. If 0/6 or 1/6 DLTs are observed, declare the regimen safe and as the maximum tolerated dose combination (MTD) of INCB024360 in combination with the vaccine. If at least 2/3 or 2/6 DLTs are observed in Cohort 1, stop and de-escalate INCB024360 to 100 mg BID in Cohort -1. If at least 2/3 or 2/6 DLTs are observed in Cohort -1, stop and de-escalate INCB024360 to 50 mg BID in Cohort -2. • **Phase IIb:** • The Phase IIb component of this trial will consist of a randomized parallel design of n=25+25=50 patients =50 patients with treatment arms: (i) DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC alone and (ii) INCB024360 in combination with the DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC. Stratification will be on clinical site (Roswell Park, U. of Pittsburgh). The primary endpoint will be progression free survival. Baseline is defined as the time following the treatment free interval, which can span 0 - 2 months, as described in Iasonos et al.[1] The primary analysis will be carried forth using a Cox proportional hazards model with factors corresponding to treatment and a continuous covariate adjustment for the length of the treatment free interval and a stratification factor for clinical site. • **Phase I/IIb:** The secondary analysis of continuous immunological response endpoints [antibody titers; NY-ESO-1 specific $CD8^+$ and $CD4^+$ frequency and function (IFN-γ ELISPOT and tumor recognition by CTL)] will be analyzed in a straightforward analysis-of-covariance (ANCOVA) fashion modeling post-treatment levels as a function of pre-treatment levels with factors corresponding to INCB024360 (yes/no) and DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC (yes/no). • **Exploratory Cohort:** The analysis of continuous immunological response endpoints (e.g., antibody titers; NY-ESO-1 specific CD8+ and CD4+ frequency and function; frequency of memory T cell populations; TCR avidity) will be analyzed via an analysis-of-covariance (ANCOVA) fashion modeling post-treatment levels as a function of pre-treatment levels with factors corresponding to Sirolimus (yes/no) and DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC (yes/no).

ROSWELL PARK CANCER INSTITUTE

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: (Network sites use subject initials) :

_____

Medical Record No.: (Network sites use subject ID) :

_____

Title: A Phase I/IIb Study of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC in Combination with INCB024360 for Patients in Remission with Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma Whose Tumors Express NY-ESO-1 or LAGE-1 Antigen

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Eligible patients will be women with epithelial ovarian, fallopian tube, or primary peritoneal carcinoma after chemotherapy with no evidence of disease or minimal residual disease for primary or recurrent disease. This may or may not be measurable. These patients would normally enter a period of observation after standard management.	
☐	☐	☐	2. Any HLA type. (Historic HLA typing is permitted.)	
☐	☐	☐	3. Tumor expression of NY-ESO-1 or LAGE-1 by IHC and/or RTPCR.	
☐	☐	☐	4. Life expectancy > 6 months.	
☐	☐	☐	5. Hematology and biochemistry laboratory results within the limits normally expected for the patient population, without evidence of major organ failure. Have the following clinical laboratory values: • ANC ≥ 1,000/μL • PLT ≥ 100,000/μL • Hgb ≥ 8 g/dL • Total bilirubin ≤ 1.5 x ULN • Serum aspartate aminotransferase (SGOT/AST) or serum alanine aminotransferase (SGPT/ALT) ≤ 3 x ULN • Serum creatinine ≤ 2 x ULN	
☐	☐	☐	6. Have been informed of other treatment options.	
☐	☐	☐	7. Age ≥ 18 years of age.	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	8. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	
☐	☐	☐	9. Have an ECOG Performance Status of ≤ 2. Refer to **Appendix A**.	
☐	☐	☐	10. The ability to swallow and retain oral medication.	
☐	☐	☐	11. Patients of child-bearing potential must agree to use acceptable contraceptive methods (e.g., double barrier) during treatment.	
☐	☐	☐	12. Patients may have received previous NY-ESO-1 vaccine therapy. Patients who received maintenance paclitaxel or bevacizumab are eligible for enrollment provided they have discontinued therapy (at least 4 weeks for prior taxane or prior bevacizumab) prior to randomization and recovered from toxicities to less than Grade 2.	

Investigator Signature: _____ **Date:** _____

ROSWELL PARK CANCER INSTITUTE

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: (Network sites use subject initials) :

_____

Medical Record No.: (Network sites use subject ID) :

_____

Title: A Phase I/IIb Study of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC in Combination with INCB024360 for Patients in Remission with Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma Whose Tumors Express NY-ESO-1 or LAGE-1 Antigen

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Metastatic disease to the central nervous system for which other therapeutic options, including radiotherapy, may be available.	
☐	☐	☐	2. Other serious illnesses (e.g., serious infections requiring antibiotics, bleeding disorders).	
☐	☐	☐	3. History of severe autoimmune disorders requiring use of steroids or other immunosuppressives.	
☐	☐	☐	4. Concomitant systemic treatment with chronic use (based on the investigator's judgement) of corticosteroids, anti-histamine or non-steroidal anti-inflammatory drugs, and other platelet inhibitory agents (See also **Section 6.4** for restrictions/recommendations on ancillary therapy).	
☐	☐	☐	5. Chemotherapy, radiation therapy, or immunotherapy within 4 weeks prior to first dosing of study drug (6 weeks for nitrosoureas). Concomitant hormonal therapies for breast cancers are allowed.	
☐	☐	☐	6. Subjects being treated with a monoamine oxidase inhibitor (MAOI), or drug which has significant MAOI activity (e.g., meperidine, linezolid, methylene blue) within 3 weeks prior to screening.	
☐	☐	☐	7. Subjects who are currently receiving therapy with a potent CYP3A4 inducer or inhibitor (e.g. clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir).	
☐	☐	☐	8. Use of UGT1A9 inhibitor including: diclofenac, imipramine, and ketoconazole.	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	9. Participation in any other clinical trial involving another investigational agent within 4 weeks prior to first dosing of study drug.	
☐	☐	☐	10. Known hepatitis B, hepatitis C, or HIV.	
☐	☐	☐	11. Mental impairment that may compromise the ability to give informed consent, and comply with the requirements of the study.	
☐	☐	☐	12. Lack of availability of a patient for immunological, and clinical follow-up assessment.	
☐	☐	☐	13. Evidence of current drug or alcohol abuse or psychiatric impairment, which in the Investigator's opinion will prevent completion of the protocol therapy or follow-up.	
☐	☐	☐	14. Pregnant or nursing female patients.	
☐	☐	☐	15. Unwilling or unable to follow protocol requirements.	
☐	☐	☐	16. Any condition which in the Investigator's opinion deems the patient an unsuitable candidate to receive study drug (i.e., any significant medical illness or abnormal laboratory finding that would, in the investigator's judgment, increase the patient's risk by participating in this study).	
☐	☐	☐	17. Known hypersensitivity to any of the study drugs that will be given to the participant.	
☐	☐	☐	18. Additional exclusion criteria for exploratory cohort ONLY: Known pulmonary hypertension.	

Patient meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll patient in study.

Investigator Signature: _____ Date: _____

Investigator Printed Name:_____

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IN-TEXT TABLES

APPENDICES

1 BACKGROUND

1.1 Study Disease: Epithelial Ovarian, Fallopian Tube, or Peritoneal Cancer

Ovarian cancer is the leading cause of death from gynecologic malignancies [2]. More than 23,000 cases occur annually in the United States, and 14,000 women can be expected to succumb to the disease [3]. Despite advances in surgery, and chemotherapy over the past 20 years, only modest progress has been made in improving the time to disease progression [4, 5], and the overall survival for patients with ovarian cancer has not changed significantly. Although the majority of women with advanced stage ovarian cancer respond to first-line chemotherapy, most of these responses are not durable and more than 70% of patients die of recurrent disease within 5 years of diagnosis. By contrast, patients with early stage ovarian cancer have excellent prognosis with currently available treatment modalities. The poor survival in advanced disease is due both to late diagnosis and lack of effective second-line cytotoxic therapy for the majority of patients who relapse following initial clinical complete response to platinum-based chemotherapy. The inability to "cure" ovarian cancer is attributed to intrinsic or acquired resistance to drug therapy which is combated with high-dose chemotherapeutic regimens complicated by significant drug related morbidity and mortality. Moreover, effective strategy for the prevention of recurrence in complete responders is lacking. Therefore, new treatment modalities and paradigms are needed before a significant impact can be expected in the prognosis of women diagnosed with epithelial ovarian cancer. Fallopian tube and primary peritoneal cancers are clinically and biologically similar to ovarian cancer, and are treated in the same fashion.

1.2 NY-ESO-1 as a Target for Immunotherapy

There is increasing evidence that the immune system has the ability to recognize tumor-associated antigens expressed in human malignancies and to induce antigen-specific humoral and cellular immune responses to these targets. Importantly, evidence from correlative human studies indicate that the presence of tumor-infiltrating lymphocytes may be associated with improved clinical outcome in several human cancers, including ovarian, melanoma, colon, gastric, liver, brain, lungs, kidney and prostate cancers. In addition, encouraging results from large-scale clinical trials of immune system-provoking therapies [1-4] have re-kindled the promise of harnessing the power of the immune system to attack cancers. Therefore, the development of strategies to enhance the potential of tumor antigen-specific CD8+ T cells and CD4+ T cells is urgently needed for extending remission rates in several cancer types. In this regard, cancer-testis antigens, a unique class of antigens that demonstrate high levels of expression in adult male germ cells but generally not in other normal adult tissues and aberrant expression in a variable proportion of a wide range of different cancer types, are promising candidates for immunotherapy. Among cancer-testis antigens, NY-ESO-1 is one of the most spontaneously immunogenic tumor antigens described so far [5, 6]. The NCI antigen prioritization panel has recently ranked NY-ESO-1 in the top 10 antigens for further development of human cancer vaccines [7].

The NY-ESO-1 antigen was initially identified by serological analysis of recombinant cDNA expression libraries (SEREX), using tumor mRNA and autologous serum from a patient with squamous cell carcinoma of the esophagus. The full-length NY-ESO-1 cDNA was cloned, encoding a protein of 180 amino acids [5]. The function of this protein is unknown. The gene

encoding NY-ESO-1 has been mapped to chromosome Xq28 [5]. A gene LAGE-1, encoding an antigen closely related to NY-ESO-1, has also been mapped to chromosome Xq28 [8].

NY-ESO-1 showed restricted mRNA expression in normal tissues, with high-level mRNA expression found only in the germ line cells in testis [5, 9]. Reverse transcription-polymerase chain reaction (RT-PCR) analysis showed NY-ESO-1 mRNA expression in a variable proportion of a wide array of human cancers, including melanoma, breast cancer, prostate cancer, lung cancer, hepatocellular carcinoma, ovarian cancer, thyroid cancer and bladder cancer [9]. Expression of LAGE-1 parallels the expression of NY-ESO-1 in most cases [8]. The pattern of NY-ESO-1 and LAGE-1 expression indicates that NY-ESO-1 family belongs to a growing family of immunogenic testicular antigens that are aberrantly expressed in a wide range of human cancers. These antigens, initially detected by either cytotoxic T cells (MAGE, BAGE, GAGE-1) or antibodies [HOM-MEL-40 (SSX2), NY-ESO-1], represent a pool of antigenic targets for cancer vaccination [10]. The expression of these genes in many different types of cancer suggests that immunization against the antigens encoded by these genes could be applied to a large proportion of cancer patients.

NY-ESO-1 has 2 characteristics that make it a viable candidate as a tumor vaccine; [1] testis-restricted expression in normal tissues and; [2] its immunogenicity. The first characteristic also applies to MAGE and other genes with a similar expression pattern (see above). A serological survey has shown that patients with melanoma, ovarian cancer, lung cancer, or breast cancer produced antibodies against NY-ESO-1 more often (19 of 209) than antibodies against MAGE-1 (3 of 209) or MAGE-3 (2 of 209). Antibodies were not detected in 70 healthy blood donors [11]. This is evidence for NY-ESO-1 immunogenicity in patients with cancer, supporting the expectation that immunity can be enhanced or induced by vaccination.

1.3 NY-ESO-1 and Ovarian Cancer

Data from 8 ovarian tumors by Chen *et al.* [6] showed aberrant expression of NY-ESO-1 in 25% (2 of 8) of epithelial ovarian cancers. Using a combination of RT-PCR and immunohistochemistry, data indicates aberrant expression of NY-ESO-1 in up to 43% (82 of 190) of patients with epithelial ovarian cancer [7]. In the current protocol, patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, primary peritoneal, and fallopian tube tumors will be candidates for antigen-specific immunotherapy.

1.4 LAGE-1 and Ovarian Cancer

The LAGE-1 cancer/testis antigen was identified by using representational difference analysis comparing testicular versus nontesticular mRNA from a melanoma patient [8]. The antigen is aberrantly expressed in a wide variety of tumors including melanomas, sarcomas, breast, lung, head and neck, prostate and bladder cancers [8]. The 180 amino acid protein encoded by the fully spliced LAGE-1 mRNA shares 94% nucleotide and 89% amino acid homology to NY-ESO-1 [8, 9]. Using RT-PCR analysis, we have found aberrant expression of LAGE-1 in 22/107 (21%) of epithelial ovarian tumors [7]. Since many NY-ESO-1 epitopes are also shared by LAGE-1, in the current protocol, patients with LAGE-1 expressing epithelial ovarian, primary peritoneal, and fallopian tube tumors will also be candidates for immunotherapy.

1.5 Clinical Studies with NY-ESO-1 Vaccines

In the first human study of NY-ESO-1 vaccination, ESO157–165 peptide in conjunction with granulocyte/macrophage colony-stimulating factor was shown to induce HLA-A2-restricted CD8+ T cell responses in patients without preexisting NY-ESO-1 immunity [12], although these peptide-induced CD8+ T cell responses were generally of low affinity and did not recognize naturally processed NY-ESO-1 [13]. Subsequently, recombinant NY-ESO-1 protein in a saponin-based adjuvant (ISCOMATRIX) was used to immunize Stage III and Stage IV melanoma patients after tumor resection [14]. More recently, patients with a range of tumor types were immunized with recombinant vaccinia NY-ESO-1 and recombinant fowlpox NY-ESO-1 [15], and recombinant protein with CpG adjuvant [16]. These vaccine strategies were found to be safe, and induced high-titered NY-ESO-1 Ab, CD4+, and CD8+ T cell responses in a high proportion of patients.

A number of studies targeting NY-ESO-1 have been conducted at Roswell Park. Protocol RP02-28, a Phase I clinical trial of immunization with an NY-ESO-1 derived long peptide of dual MHC Class I/II specificities, mixed with incomplete Freund's adjuvant (Montanide ISA51) in 18 ovarian cancer patients [17]. Protocol I 13303, a Phase II clinical trial of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in ovarian cancer patients whose tumors express NY-ESO-1 or LAGE-1. One serious adverse event (SAE) was reported to be unlikely due to rV-NY-ESO-1 or rF-NY-ESO-1. Toxicity and immunological results are currently being evaluated. Protocol I 27008 is an on-going Phase I study testing epigenetic modulation of NY-ESO-1 with 5-Azacytidine in combination with recombinant NY-ESO-1 protein admixed with GMCSF and IFA in ovarian cancer patients receiving liposomal doxorubicin for recurrent disease. Nine out of 12 planned patients have been enrolled; and no treatment related Grade 3 adverse events (AEs) have been noted.

Protocol I 125207, a Phase I study testing (ALVAC)-NY-ESO-1/TRICOM (recombinant canarypox expressing NY-ESO-1 and a triad of co-stimulatory molecules, TRICOM). Planned accrual of 12 patients has been completed and no Grade 3 toxicities attributable to the vaccine were noted. Collective experiences from these studies indicate that NY-ESO-1 vaccines are safe. Moreover, NY-ESO-1 specific antibody responses and/or CD8+ and CD4+ T cell responses were induced by vaccinations in a high proportion of patients. Vaccine-induced CD8+ and CD4+ T cell clones were shown to recognize NY-ESO-1 -expressing tumor targets. However, inhibitory networks such as the indole-amine 2,3 dioxygenase (IDO), PD-1/PDL-1, CTLA4 pathways act to restrict the efficacy of anti-tumor immune responses.

Recently, protocol I191511 "A Phase I Clinical Trial of mTOR Inhibition With Rapamycin for Enhancing Intranodal Dendritic Cell Vaccine Induced Anti-Tumor Immunity In Patients With NY-ES0 -1 Expressing Solid Tumors" was completed and the 4 mg/day Sirolimus dosing was tolerated well. This same dose is proposed in the exploratory cohort of this protocol.

1.6 Study Drugs

1.6.1 DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) with Adjuvant Poly-ICLC

The investigational vaccine is composed of DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC.

1.6.1.1 DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401)

The DEC-205 receptor has been shown to be an efficient mAb-based target to enhance the induction of strong Ag-specific immune responses and cross-presentation in mice [10] and humans [11]. The receptor is also expressed on human monocyte-derived dendric cells along with other endocytic receptors, such as the mannose receptor/CD206 and DC-SIGN/CD209 (reviewed in ref[12]). In order to enhance the efficiency of NY-ESO-1 presentation to $CD4^+$ and $CD8^+$ T cells *in vivo*, exogenous NY-ESO-1 protein will be targeted to the DEC-205 endocytosis receptor on dendric cells for cross-presentation in this clinical trial. In a recent study, full-length NY-ESO-1 was fused to the C terminus of human anti-DEC-205 (DEC205mAb-NY-ESO-1 fusion protein). Whereas non-targeted and Ab-targeted NY-ESO-1 proteins similarly activated $CD4^+$ T cells, cross-presentation to $CD8^+$ T cells was only efficiently induced by targeted NY-ESO-1 [13]. In addition, DEC-205 targeting elicited specific $CD4^+$ and $CD8^+$ T cells from PBLs of cancer patients. In this study, the DEC-205 mAb-NY-ESO-1 fusion protein will be directly injected intracutaneously in an effort to prime endogenous dendric cells with full length NY-ESO-1 protein and thereby prime specific $CD4^+$ and $CD8^+$ T cells to NY-ESO-1.

DEC205mAb-NY-ESO-1 fusion protein has been developed by Celldex Therapeutics, Inc, under the code designation CDX-1401. CDX-1401 is a fusion protein consisting of a fully human monoclonal antibody (HuMab) of IgG1 (kappa) isotype with specificity for the DC receptor, DEC-205, genetically linked to the full length NY-ESO-1 tumor antigen (Ag) protein (10). A detailed discussion of the preclinical pharmacology, pharmacokinetics, and toxicology of Anti DEC-205-NYESO-1 Fusion Protein can be found in the Investigator's Brochure.

1.6.1.2 Clinical Studies with CDX-1401 and Adjuvants

As of February 4, 2011, safety data are available for 21 patients who have received CDX-1401 (0.1 mg, 1 mg or 3 mg) plus topical resiquimod (0.5 mg; delivered as 250 mg of 2% gel) or poly ICLC (2 mg flat dose given SQ). There have been no dose-limiting toxicities, treatment-related Grade 3 or Grade 4 AEs, treatment-related SAEs or AEs requiring discontinuation of treatment. Injection site reactions, all Grade 1 in severity, have occurred in 76% of treated patients. These reactions have included rash, erythema, pruritus, pain, induration and swelling. Additional treatment-related toxicities have all been mild to moderate in severity, and have included, fatigue (24%), decreased appetite (14%), nausea (10%), arthralgia (10%), headache (10%) and pruritus (10%). Six patients with stable disease (range: 4.7 to 11.5 + months) have been retreated for more than one cycle, including 4 who received ≥ 3 cycles. Poly-ICLC has been the adjuvant chosen for further studies of this protein based upon preliminary data on induction of anti-NY-ESO-1 specific immune responses.

1.6.1.3 POLY-ICLC

Poly-ICLC (Hiltonol, Oncovir by Dalton, Inc. 3203 Cleveland Ave, NW Washington, DC, 20008) is an experimental viral mimic and broad activator of innate immunity. While initially developed as an interferon inducer, poly-ICLC has much broader biological effects in humans, including specific antiviral, immune activating, vaccine adjuvant, and antitumor actions.

Synthetic double stranded RNA (dsRNA) such as poly-IC, which consists of a pair of strands of poly-inosinic and poly-cytidylic acids, are not normally found in mammalian cells, but they are the basic genetic material or are replication byproducts of many viruses (29). Plain poly-IC itself proved to be ineffective in primates because it is rapidly inactivated by natural enzymes in the blood. However, stabilized poly-IC with poly-lysine is a very stable dsRNA that is a potent interferon inducer in man. Early, short term, high dose cancer trials showed that high dose poly-ICLC could induce very large amounts of interferon production in man, but with only modest therapeutic effects and moderate transient toxicity (29). Low dose poly-ICLC is a potent clinical activator of a variety of host defense mechanisms that go well beyond simple induction of interferons and which include reversal or preemption of certain viral or tumor induced inhibitions, broad immune stimulation, gene regulatory and specific antiviral, and anticancer effects, with little or no toxicity (29,30).

In this study low dose poly-ICLC will be used as a direct immune-enhancer in order to activate T-cells, natural killer cells, and, dendritic cells, release of cytokines such as interferons, interleukins, corticosteroids, and tumor necrosis factor (TNF). Its recently demonstrated effect on dendritic cells through Toll-like receptors (TLR3) may be especially important since CDX-1401 directly targets these cells (31). As described above, dendritic cells play a critical role in the immune response by recognizing pathogens and presenting their antigens to the immune system. The poly-ICLC used in this study will provide an adjuvant effect for the CDX-1401 and lead to increased antibody and cellular immune response to NY-ESO-1antigen found on antigen-presenting cells.

1.6.2 INCB024360 (4-amino-1,2,5-oxadiazole-3-carboximidamide) Inhibitor of Indoleamine 2,3 Dioxygenase (IDO)

Recent interest has focused on the role of indoleamine 2,3–dioxygenase (IDO) as a mechanism of induction of tolerance to malignancy. IDO is a heme-containing, monomeric oxidoreductase that catalyzes the degradation of the essential amino acid tryptophan (Trp) to N-formylkynurenine (Kyn). Kynurenine can be subsequently metabolized through a series of enzymatic steps to NAD+. IDO is the first rate-limiting enzyme in one of the breakdown pathways of Trp. In an alternative pathway for Trp metabolism, Trp hydroxylase leads to the formation of serotonin and melatonin.

The expression and activity profiles of IDO are distinct from those of Trp dioxygenase (TDO), an enzyme predominantly expressed in liver that catalyzes the same enzymatic reaction as IDO and maintains proper Trp balance in response to dietary uptake. In contrast to TDO, IDO is expressed in a variety of tissues, with particularly high levels found in areas of contact with potential sources of immune challenge (e.g., gut, respiratory tract, placenta, spleen), consistent with a role for regulating Trp metabolism in a local microenvironment. The IDO-driven oxidation of Trp results in a strongly inhibitory effect on the development of T cell mediated responses by blocking T cell

activation and inducing T cell apoptosis. Both the reduction in local Trp levels and the production of Trp catabolites that are inhibitory to cells contribute to the immunosuppressive effects [14].

IDO activity also promotes the differentiation of naïve T cells to cells with a regulatory phenotype (T_{reg}) [15]. Since increased T_{reg} activity has been shown to promote tumor growth and T_{reg} depletion has been shown to allow an otherwise ineffectual anti-tumor immune response to occur [16], IDO expansion of T_{regs} may provide an additional mechanism whereby IDO could promote an immunosuppressive environment.

Preliminary data clearly supports the role of IDO in contributing to the profound immune suppressive environment in ovarian cancer. There are currently 2 on-going clinical trials testing IDO inhibition in patients with solid tumors (NCT01792050; NCT01685255, http://www.cancer.gov/search/ViewClinicalTrials). One trial is testing the IDO inhibitor, dextro - 1-methyl-tryptophan (D1-MT) in combination with chemotherapy. In contrast to the levo-isoform (L-INCB024360) that blocks IDO1, D1-MT, which is used in the clinical trials inhibits IDO2, and exhibits little IDO blockade in some *in vitro* test systems [17]. Emerging data indicate that IDO2 is NOT affected by D-1-MT [18, 19]. In studies conducted at Roswell Park, it has been found that, L-1-MT and D/L-1-MT can restore proliferation of tumor and peripheral blood T cell subsets of ovarian cancer patients, D-1-MT does not effectively restore IDO-induced arrest of human T cell proliferation [20]. Given the controversy surrounding D-1MT, we have focused on INCB024360 (4-amino-1,2,5-oxadiazole-3-carboximidamide), a novel, potent and selective inhibitor of IDO1 enzyme activity [21, 22]. INCB024360 was recently identified by high throughput screening and was shown to be a reversible and competitive inhibitor of IDO1, resulted in efficient and durable suppression of IDO1 activity in mice to levels seen in *Ido1*-deficient mice, and impedes tumor growth in a dose- and lymphocyte-dependent fashion [21, 23, 24].

Published literature and preliminary data indicate that INCB024360 is the most potent inhibitor of human IDO1 reported to date, more than 10,000-fold more potent than 1-methyl-tryptophan when tested in the same assays. The compound also exhibits more than 100-fold selectivity over TDO, IDO2, and tryptophan transporters [22]. Moreover, INCB024360 significantly promotes proliferation, survival, and functions of T cells and abrogates generation of $CD4^+$ T_{regs}.

Extensive preclinical pharmacologic, pharmacodynamic, pharmacokinetic and toxicology studies have been performed with INCB024360. There is emerging clinical data on the administration of INCB024360 in humans. Consequently, any information about specific risks and the potential for AEs is based on emerging results from an on-going Phase I study.

1.6.2.1 INCB024360 Preclinical Pharmacology

1.6.2.1.1 *In Vitro* Pharmacology

INCB024360 represents a novel, potent, and selective inhibitor of the enzyme IDO. In cell-based assays, INCB024360 potently inhibits IDO in both human tumor cells and human dendritic cells resulting in reduced Trp to Kyn conversion (IC50 values = 7.1 nM - 12.7 nM). INCB024360 does not significantly inhibit other proteins that could impact Trp catabolism and demonstrates selectivity against a broad panel of G-protein coupled receptors (GPCRs), ion channels, transporters and enzymes. As described above, IDO driven oxidation of Trp results in a strong

inhibitory effect on the development of T cell mediated responses by blocking T cell activation and inducing G1 arrest and apoptosis. INCB024360 reverses the inhibition of the development of T cell mediated responses that IDO activity imparts, resulting in enhanced T cell and NK cell proliferation, enhanced interferon-gamma (IFN-γ) production, reduced T_{reg} differentiation, reduced DC apoptosis and enhanced expression of DC activation markers. INCB024360 reversal of the IDO-mediated suppression of T cell proliferation is dose-dependent with a potency consistent with its inhibition of Trp to Kyn conversion (EC50 = 17.7 nM). *Ex vivo*, INCB024360 inhibits IFN-γ induced IDO activity in human whole blood with an IC50 value of 125 nM ± 26 nM. This value is consistent with the predicted value of 130 nM, based on the *in vitro* potency in cell-based assays and the measured free fraction of INCB024360 in human serum.

1.6.2.1.2 *In Vivo* Pharmacology

The *in vivo* data demonstrate that INCB024360 can inhibit IDO systemically and, importantly, in tumors and tumor draining lymph nodes. INCB024360 was efficacious in multiple mouse models of cancer and its ability to reduce tumor growth was dependent on a functional immune system – consistent with its proposed mechanism of action. Moreover, INCB024360 enhanced lymphocyte function in tumors and tumor draining lymph nodes. Finally, INCB024360 improved the tumor growth control of cytotoxic chemotherapy when used in combination.

In order to examine regulation of IDO activity by INCB024360 *in vivo*, naïve C57BL/6 mice were administered a single oral dose of INCB024360 and blood samples were analyzed to determine the amount of Kyn and INCB024360 in plasma over time. Plasma Kyn levels decreased within 1 hour and stayed at their minimum levels for as long as plasma levels of INCB024360 exceed the mouse protein binding adjusted EC50 of 1.2 = μM (approximately 8 hours). Consistent with specific inhibition of IDO by INCB024360, no reduction in Kyn levels were observed in mice lacking IDO expression even though compound exposures were similar between mouse strains. These data suggest that monitoring Kyn levels in plasma may be a useful pharmacodynamic marker of the activity of INCB024360.

Orally dosed INCB024360 was evaluated in immune competent and compromised mouse tumor models to characterize the effects of IDO inhibition on tumor growth and to determine if any observed effect was mediated by the immune system. Balb/c mice bearing well established subcutaneous syngeneic CT26 colon carcinomas, which had previously been demonstrated to express IDO, were treated orally with INCB024360. As a single agent, INCB024360 inhibits CT26 tumor growth in a dose dependent fashion, with 46% and 66% tumor growth control (TGC) at doses of 30 mg/kg and 100 mg/kg, respectively. Similar results were seen in the PAN02 pancreatic cancer model in C57BL/6 mice, with twice daily oral doses of INCB024360 of 25, 50, or 100 mg/kg resulted in 61%, 63% and 83% TGC, respectively. Consistent with the proposed mechanism, there was no effect of 30 mg/kg or 100 mg/kg INCB024360 on the growth of CT26 tumors in immunocompromised Balb/c nu/nu mice. These data imply that INCB024360 can impede tumor growth as a single agent and indicate that T cells are required for antitumor activity.

In summary, pharmacological data obtained in both *in vitro* and *in vivo* model systems support the potential utility of orally administered INCB024360 in the treatment of malignant disease.

1.6.2.2 INCB024360 Preclinical Pharmacokinetics

The *in vitro* permeability of INCB024360 was considered moderate. In the presence of various P-glycoprotein inhibitors, the bi-directional transport ratio was partially decreased suggesting that INCB024360 is likely a P-glycoprotein substrate and perhaps other apically localized transporters. In single oral dose pharmacokinetics studies in mice, rats, cynomolgus monkeys, and dogs, the T_{max} values for INCB024360 ranged from 0.25 hours to 2.0 hours. The absolute oral bioavailability ranged from low to moderate and was consistent with the moderate clearance and the limited aqueous solubility of INCB024360 (< 100 μg/mL). In mice, rats, cynomolgus monkeys, and dogs, the apparent steady-state volume of distribution values of INCB024360 suggested that INCB024360 is distributed beyond the total volume of body water in mice, rats, and monkeys while apparently limited to body water in dogs. The reason for lower volume of distribution in dogs relative to the other species studied is not clear. In rats, INCB024360 exhibits limited penetration across the blood-brain barrier.

The *in vitro* serum and plasma protein binding of INCB024360 in mice and dogs was high with mean unbound fractions ranging from 3.1% to 4.1%. The *ex vivo* unbound fraction determined in plasma from mice and dogs given INCB024360 was 2.5% and 3.8%, respectively. The mean *in vitro* unbound fraction of INCB024360 in human plasma was 3.1%, and the compound binds predominantly to human serum albumin.

The potential for INCB024360 to inhibit human cytochrome P450s was studied *in vitro*. The inhibitory IC50 values for INCB024360 were > 25 μM for CYP1A2, CYP2B6, CYP2C8, CYP2C9, and CYP2D6 while the IC50 values for CYP2C19 and CYP3A4 were greater than and equal to 12.5 μM, respectively. Based on this data, the potential for INCB024360 to cause drug-drug interactions through CYP inhibition is considered low. In addition, the potential for INCB024360 to induce CYP3A isozymes was studied *in vitro* using the human pregnane X receptor reporter gene assay, and results show that the potential for INCB024360 to induce CYP3A4 in clinical studies is low.

INCB024360 exhibits low to moderate intrinsic clearance *in vitro* following incubation with hepatic S9 fractions prepared from mice, rats, dog, cynomolgus monkeys, and humans. Consistent with the *in vitro* data, INCB024360 exhibited moderate systemic clearance (ranging from 24% to 58% of hepatic blood flow) in pharmacokinetic studies. Based on studies with recombinant CYP isozymes, INCB024360 is metabolized primarily by CYP3A4 *in vitro*. A major route of INCB024360 *in vivo* metabolism is *via* glucuronidation. Based on studies with recombinant UGT isozymes, INCB024360 is metabolized by UGT1A9 *in vitro*. Glucuronidation is the proposed primary metabolic pathway in human based on *in vitro* studies. After IV administration, INCB024360 had a moderate half-life in mice (2.5 hours), rats (male 1.4 hours, female 1.7 hours), monkeys (3.3 hours), and dogs (3.1 hours). In single intravenous dose pharmacokinetics studies in mice, monkeys, and dogs, less than 3% of INCB024360 was excreted as parent in urine.

1.6.2.3 INCB024360 Preclinical Safety

INCB024360 was studied in a variety of *in vitro* binding and enzyme assays against a panel of receptors, channels, transporters, and non-kinase enzymes to evaluate its specificity. INCB024360 demonstrated cross reactivity at the vasopressin 1a receptor, with an IC50 value of 0.67 μM. Weak

activity was also noted for the dopamine transporter (71% inhibition at a concentration of 10 μM) and for carbonic anhydrase II (IC50 5.3 μM). Given that INCB024360 is a potent inhibitor of IDO with an IC50 value of 72 nM, the risk of unintended pharmacological activity is expected to be low. Evaluation of the potential interaction of INCB024360 with the hERG channel was evaluated using voltage-clamped human embryonic kidney cells (HEK293). There was no significant inhibition of the hERG potassium current at 10 μM.

A preliminary assessment of the potential genotoxicity of INCB024360, using a non-GLP bacterial mutagenicity assay, with and without metabolic activation, indicated that INCB024360 lacks the potential to be genotoxic. Oral administration studies in both mice and beagle dogs were conducted in order to assess the potential systemic toxicity of INCB024360. In single dose studies in mice, no mortality or adverse changes in parameters evaluated were noted at doses up to 4000 mg/kg. Single doses of up to 540 mg/kg were well tolerated in the dog.

Toxicological data from 2 repeat oral dose administration studies in the mouse (8-day non-GLP and 28-day GLP) revealed no adverse findings in any parameter evaluated. The NOEL for the 28-day study in mice was defined as 2000 mg/kg/day. Two repeat dose oral administration studies were conducted in the dog (7-day non-GLP and 28-day GLP). All dose levels in both studies were well tolerated, resulting in the NOAEL being identified as 500 mg/kg/day. INCB024360-related changes included an increased incidence of diarrhea in 500 mg/kg/day dogs as well as sporadic emesis. In dogs, pharmacodynamic analysis determined that all dose levels used in the 28-day study were pharmacologically active with almost 100% inhibition of tryptophan conversion to kynurenine being achieved at 1 hour post-dose for all dose levels.

The projected clinical dose is 550 mg QD, a dose estimated to be associated with achieving IC90 at trough. The projected unbound AUC associated with this dose is 0.72 μM*h. Safety margins calculated by comparing the clinical projected AUC versus the unbound no-observed-adverse-effect level (NOAEL) dose of 500 mg/kg/day in the dog 28-day study are 11- and 17-fold for male and female dogs, respectively. For the unbound no-observed-effect level (NOEL) dose of 2000 mg/kg/day in the mouse, the safety margins are 20- and 37-fold for male and female mice, respectively. Based on the planned clinical starting dose of 50 mg, the lack of any apparent INCB024360-related toxicity and the large safety margins associated with the predicted clinically efficacious dose, the potential risk associated with clinical administration of INCB024360 is expected to be low.

1.6.2.4 INCB024360 Clinical Studies

INCB24360-101 has been evaluated in Phase I study conducted by Incyte Corporation in subjects with refractory solid tumors. The study has used a 3+3 dose escalation design, with doses of INCB024360 ranging from 50 mg once daily (QD) to 700 mg twice daily (BID). Three subjects have initiated treatment at 50 mg QD, 4 subjects at 50 mg BID, 3 subjects at 100 mg BID, 6 subjects at 300 mg BID, 11 subjects at 400 mg BID, 5 subjects at 500 mg BID and 4 subjects at 700 mg BID.

INCB024360 has generally been well tolerated. The most common Grade 1 or Grade 2 AEs in ≥ 20% reported have been fatigue, nausea, decreased appetite, vomiting, constipation, abdominal pain, diarrhea, dyspnea, back pain, and cough. The most common Grade 3 or Grade 4 AEs were

abdominal pain, hypokalemia, and fatigue (9.6% each). Despite 1 patient reporting a dose-limiting toxicity (DLT) of radiation pneumonitis at 300 mg BID, additional subjects were enrolled at this dose and no additional DLTs were reported. At 400 mg BID, 1 patient reported a DLT of Grade 3 fatigue, with no additional DLTs at this dose level. Another patient at 400 mg BID developed asymptomatic and transient hypopituitarism after 1 cycle of therapy and was able to continue dosing with INCB024360. Among the 18 subjects treated at either 600 mg or 700 mg BID, no DLTs were observed. Dose escalation was completed based on preliminary pharmacokinetic and pharmacodynamic data, which demonstrated that doses of INCB024360 above 300 mg BID achieved exposures exceeding the IC90 determined in nonclinical models and, in pharmacodynamic assays, reached a plateau for Kyn/Trp levels and ratios. In the final cohort of 600 mg BID, the effect of food on INCB024360 absorption was evaluated. No objective responses were reported. At 2 months, stable disease was seen in 15 patients and lasted ≥ 4 months in 7 patients. The main AE reported in subjects receiving more than 2 cycles of INCB024360 has been Grade 1 or Grade 2 fatigue.

In a combination study conducted by Incyte Corporation of INCB024360 and ipilimumab in metastatic melanoma patients, 5 of 7 subjects enrolled at a dose of 300 mg BID of INCB024360 plus 3 mg/kg of ipilimumab, developed Grade 3 or Grade 4 AST and ALT elevations between 2 and 4 cycles of treatment. Only one of these subjects had a bilirubin elevation of Grade 1.

Plasma levels of INCB024360 and plasma levels of Kyn were measured in serial samples. Steady-state linear pharmacokinetics were obtained on or before Day 8 and there was achievement of steady-state on Day 15. The half-life was 2.3 to 4 hours. Pharmacodynamic correlates suggest that significant target inhibition was achieved at BID doses greater than or equal to 300 mg BID, resulting in steady state exposures that exceeded the IC90. Blood samples for tryptophan and kynurenine levels as a pharmacodynamic effect show significant target inhibition with doses of 50 mg BID or greater. BID doses greater than or equal to 300 mg BID result in normalization of kynurenine levels in those patients who show elevations at baseline. Immunohistochemical staining of 11 patients demonstrated that IDO1 tumor expression could be observed in tumor cells and/or infiltrating inflammatory cells in 10/11 samples, and CD3+ T cells and Foxp3+ T regulatory cells were present in all of the samples examined.

In unpublished preliminary data from Incyte, Kynurenine concentrations and the Kyn/Trp ratio were higher in the serum of most patients compared to controls and normalized following treatment with inhibitor when treated with doses that exceeded the IC90 (i.e., > 300 mg BID (n = 19 patients)).

Data for All Patients

- Kynurenine concentrations:
  - Normal individuals: median, 2.0 µM
  - Cancer patients: median, 2.1 µM (1.3 µM - 4.2 µM)
  - Post inhibitor: median, 0.97 µM (0.5 µM - 1.7 µM)
- Kyn/Try ratios:
  - Normal individuals: median, 29.9 nmol/µmol

- Cancer patients: median, 37.4 nmol/mmol (17.8 - 68.9 nmol/µmol)
- Post inhibitor: median, 18.3 nmol/µmol

Data for Patients with Kyn levels > 2 µM (n = 8) or K/T > 30 nmol/µmol (n = 14)

- Kynurenine concentrations:
  - Normal individuals: median, 2.0 µM
  - Cancer patients: median, 2.7 µM (2.1 µM - 4.2 µM)
  - Post inhibitor: median, 1.1 µM (0.5 µM - 1.7 µM)
- Kyn/Try ratios:
  - Normal individuals: median, 29.9 nmol/µmol
  - Cancer patients: median, 42.0 nmol/µmol (34 – 69 nmol/µmol)
  - Post inhibitor: median, 18.6 nmol/µmol (8 - 43 nmol/µmol)

1.6.3 Sirolimus (used in Exploratory Cohort at Roswell Park ONLY)

Sirolimus is a specific inhibitor of the mammalian target of sirolimus (mTOR complex 1) and is the active metabolite of temsirolimus, which is the first in class mTOR inhibitor approved for use in RCC mTOR inhibitor approved for the treatment of renal cell cancer [25-27]. Sirolimus binds to an intracellular protein (FKBP-12), and the protein–drug complex binds to mTOR to inhibit its kinase activity [25]. In preclinical tumor models, sirolimus inhibited cell proliferation, cell growth, survival pathways, and tumor angiogenesis [28, 29]. Initial clinical studies in cancer patients investigated IV sirolimus administered at dosages that were corrected for body surface area (mg/m2) and were designed to establish tolerability, safety, and pharmacokinetic parameters [30, 31]. When administered as a 30-minute infusion once weekly, sirolimus was well tolerated over a wide range of doses (7.5 mg/m2 – 165 mg/m2) [31]. Rash and mucositis/stomatitis were the most frequent drug-related AEs, and thrombocytopenia was the main dose-limiting toxicity. Antitumor activity was observed in heavily pretreated patients, including patients with advanced RCC, who received different doses and schedules) [30, 31]. Overall, the most frequent Grade 3 or Grade 4 mTOR inhibition-related AEs (n = 110) were hyperglycemia (17%), hypophosphatemia (13%), anemia (9%), and hypertriglyceridemia (6%).

The major limitation for combining sirolimus and immunotherapy is that mTOR inhibitors are generally regarded as immunosuppressive via several mechanisms, including the induction of regulatory T-cells. Although sirolimus is thought to have immunosuppressive effects, its effects on the potential inhibition of T-lymphocyte function after repeated daily administrations in euthymic mice showed that T-lymphocyte activity recovered within 24 hours after the discontinuation of treatment.

Sirolimus has been used in patients with HIV and hepatitis undergoing solid organ transplant. In several reports no increased incidence of reactivation of HIV or any other viral infection have occurred.

Recent studies by our group and others show that mTOR inhibition appears to significantly condition tumor bearing hosts for augmented vaccine induced T-cell mediated tumor control) [32,

33]. It has been observed that mTOR inhibition selectively switches the transcriptional program in effector CD8+ T-cells from T-bet to Eomes expression. Eomes expression has been known to increase in the CD8+ precursor memory T-cells. The observations will imply that sirolimus conditions CD8+ T-cells to have greater capacity for lymph node homing and retention.

Since sirolimus is cytostatic, the potential for therapeutic synergy with immunotherapy is likely to include (1) increase the dose of antigens delivered for presentation (2) enhanced anti-tumor T-cell response in the draining lymph node through cross-priming (3) effects on T-cell trafficking (4) generation of immunological memory. Taken together, we propose that sirolimus and immunotherapeutic strategies might be combined to control tumor growth, and the vaccine-induced memory immune responses would still be available to delay time to disease relapse.

1.6.4 Potential Risks and Benefits

1.6.4.1 INCB024360 Potential Risks

There have been no adverse findings in toxicology or safety pharmacology assessments described herein. INCB024360-related changes included an increased incidence of diarrhea in 500 mg/kg/day dogs as well as sporadic emesis. Weak cross reactivity of INCB024360 to the human vasopressin 1a receptor, dopamine receptor and carbonic anhydrase II is not expected to pose a risk to humans. Blood pressure will be monitored in this clinical study and blood electrolytes will also be assessed. In 28-day toxicology studies, Cmax values have exceeded the IC50 for the IDO enzyme (72 nM) by up to 37-fold and the IC50 for the vasopressin 1a receptor by up to 4-fold (13-fold in single dose studies) in the absence of any toxicity, so the risk of unintended pharmacological activity is expected to be low.

Hypothetically, IDO represents an important immune control enzyme and cells expressing IDO are capable of suppressing amplified immune effector responses and promoting immune tolerance under various physiological conditions. Inhibition of IDO using 1-MT has been shown to induce fetal allograft rejection in mice (Munn et al, 1998). Syngeneic, pluripotent bone marrow stem cells, transferred to mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis, enhanced recovery, prevented relapses and promoted myelin repair through their expression of IDO and this was blocked by administration of 1-MT (Matysiak et al, 2008) and inhibition of IDO by systemic administration of 1-MT at clinical onset significantly exacerbated disease scores in this model (Sakurai et al, 2002). Blocking IDO with 1-MT also aggravated the severity of arthritis and enhanced the immune responses in mice with collagen-induced arthritis (Szántó et al, 2007). Taken together, these studies imply that IDO may be a negative inhibition pathway to control autoimmune diseases. For these reasons, patients with autoimmune disease are excluded from participation in this study and study participants will be closely monitored for signs or symptoms of developing autoimmune or inflammatory disease.

Recent studies with the anti-CTLA-4 antibody ipilimumab have shown dysregulation of mucosal immunity and gastrointestinal toxicity which manifests as diarrhea (Berman et al, 2008). The immune related AEs are distinct from classic inflammatory bowel diseases and graft-versus-host disease in terms of histologic features but respond to treatment with steroids. Since IDO targets

immune regulation differently from the anti-CTLA-4 antibodies, it is unknown whether similar toxicities may be observed with IDO inhibition. There is no evidence of gastrointestinal toxicity in the IDO knockout mouse.

1.6.4.2 INCB024360 Benefits

Patients may experience improvement in their disease status during the study. It is unknown if there will be significant benefit for the patients enrolled in this study.

1.6.4.3 Sirolimus Potential Risks

Sirolimus is metabolized primarily by CYP3A4 in human liver microsomes, and may influence substrate transport via P-glycoprotein. Since there are many agents that affect the metabolic activity of CYP3A4 and P-glycoprotein, there is a potential for drug-drug interactions to occur between sirolimus and co-administered drugs. Current knowledge and available data suggest caution when combining sirolimus with strong CYP3A4 inhibitors. Strong CYP3A4 inhibitors are to be avoided unless there is no alternative available. Co-administration of ketoconazole should be avoided if possible. Patients would be strongly encouraged to avoid herbal supplements including, but not limited to St. John's Wort, during active treatment. Sirolimus may inhibit CYP2D6 and potentially interact with drugs that are substrates for CYP2D6.

- **Interstitial Pneumonitis/Alveolitis**

There have been a number of reports of a non-specific interstitial pneumonitis/alveolitis among oncology patients who received sirolimus. Some are asymptomatic and detected on CT scan or chest x-ray, while symptoms such as dyspnea, cough, and fever were noted in others. Some cases resulted in discontinuation of sirolimus and treatment with corticosteroids and/or antibiotics, while others did not require intervention. In most cases to date, where outcome is known, there was improvement or resolution following such interventions. There have been some occurrences of rechallenge with subsequent recurrence of the pneumonitis. This type of event has also been reported in association with the closely-related compound sirolimus (when given orally to transplant patients) and other agents.

- **Pulmonary Hypertension**

To date, there have been sporadic reports of pulmonary hypertension in oncology patients receiving sirolimus. A causal relationship to sirolimus cannot be determined based on the available information. These reports will continue to be monitored.

- **Renal Impairment**

Renal impairment, insufficiency, and failure have been reported in some oncology patients receiving sirolimus. A causal relationship to sirolimus cannot be determined based on the available information. These reports will continue to be monitored.

- **Thrombotic Events**

Thrombotic events, including thromboembolism, thrombosis, arterial thrombosis limb, venous thrombosis deep limb, venous thrombosis, vena cava thrombosis, and pulmonary embolism, have been reported in patients receiving sirolimus. Although most events were considered unrelated to

sirolimus, it should be noted that sirolimus is associated with increased fibrinogen levels. The clinical significance of this association is not yet known.

- **Central Nervous System Bleeding**

Central nervous system (CNS) bleeding has been observed in some oncology patients receiving sirolimus. While a relationship to sirolimus is not suspected, it cannot be ruled out. Most of these patients had CNS tumors and most were receiving anticoagulants (low molecular weight heparin, warfarin, etc). It is not clear if there could have been a drug-drug interaction between sirolimus and the anticoagulant. Patients receiving anticoagulants should be monitored closely.

- **Immunosuppression with Continuous Sirolimus Dosing**

Patients treated with a continuous dose of sirolimus may have immunosuppressant. As such, these patients should be carefully observed for occurrence of infections, especially opportunistic infections.

1.7 Rationale for the Use of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) with Adjuvant Poly-ICLC in Combination with INCB024360

In previous experimental studies, we have observed that (i) the beneficial prognostic effect of $CD8^+$ tumor infiltrating lymphocytes (TILs) in ovarian cancer is modulated by $CD25^+FOXP3^+$ subpopulation of $CD4^+$ T cells with immunosuppressive properties [34] (ii) tumor and dendritic cell expression of indoleamine 2,3 dioxygenase (IDO) mediate preferential accumulation of $CD4^+CD25^+FOXP3^+$ T cells in human ovarian cancer (iii) NY-ESO-1 peptide vaccination failed to modulate the suppressive effect of T_{regs} on high-avidity NY-ESO-1-specific T cell precursors [35]. Therefore, the ideal strategy for improving anti-tumor efficacy should combine (i) abrogation of T_{reg} induction and (ii) augmentation of clonal expansion of high avidity effector T cells. In this clinical trial, we have focused on targeting a valid, immunogenic ovarian tumor antigen NY-ESO-1; along with enhancement of T cell clonal expansion and abrogation of T_{regs} with the IDO inhibitor, INCB024360.

The survival of patients with Stage III/IV ovarian cancer has been strongly correlated with the presence or absence of intratumoral T cells [34, 36]. Additionally, high levels of intratumoral IDO expression in ovarian cancer have been found to correlate with a reduced number of tumor infiltrating lymphocytes [37]. This high level of IDO expression was found to correlate with disease stage as well as with decreased survival, a finding that has been described by a number of investigators [38-41]. Thus, there is a strong rationale to utilize IDO inhibition as a strategy to restore antitumor immunity in advanced ovarian cancer.

Based on the safety profiles in Phase I and on-going Phase II studies, there is an extremely low potential for toxicity of the treatment combination. The vaccine (CDX-1401 with adjuvant polyICLC) has been shown to be safe in Phase I studies, with virtually no dose limiting toxicities, treatment-related Grade 3 or Grade 4 AEs, treatment-related SAEs or AEs requiring discontinuation of treatment. INCB024360 has been tested in Phase I trials, and is currently undergoing Phase II evaluation. INCB024360 was shown to be safe at doses as high as 600 mg bid. This is much lower than the maximum 300 mg bid that we propose in this clinical trial.

1.8 Rationale for Exploratory Cohort at Roswell Park ONLY (DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) with Adjuvant Poly-ICLC in Combination with Sirolimus)

A major limitation of current cancer vaccines is the ineffective generation of memory T-cell precursors. This issue will be addressed in the current clinical trial via the use of sirolimus to promote the generation of effector/memory T-cells.

The induction of memory T-cell responses is a key to prevent tumor recurrence, but has been a major challenge for all tumor immunotherapy approaches. Recently, we have reported that rapamycin mediated inhibition of mammalian target of rapamycin (mTOR) switches effector CD8+ T cells to memory precursor like phenotype [33]. Our data indicate that (i) inhibition of mTOR activity switches T-bet for Eomesodermin expression, facilitating transition of type 1 effector cells for memory (ii) mTOR inhibition post-immunization enhances tumor control in EOC bearing hosts (iii) rapamycin mediated mTOR inhibition is reversed over time, (iv) varying the duration (0-8, 0-20 or 0-40 days) of mTOR inhibition after immunization produces memory CD8+ T cells with varying extent of type 1 effector functions. Collectively, these findings encourage us to exploit the use of mTOR inhibition to generate durable CD8+ T cell responses and test its ability to prolong EOC remission rates in a clinical trial.

Based on our observations, we propose to test the hypothesis that mTOR inhibition will condition vaccine induced T cells for enhanced persistence, antigen recall and durable tumor immunity in a human clinical trial. We plan to use rapamycin, a clinically available mTOR inhibitor that has not been previously used in this fashion, for our “Proof-of-Principle” studies. The regimen of mTOR inhibition that can optimally combine with NY-ESO-1 protein with CDX-1401 vaccination to generate memory T-cells in cancer patients is not established. As varying the duration and timing of mTOR inhibition in relation to vaccination leads to distinct functional outcomes in T-cells, the dose and schedule for sirolimus in this study has been based on our completed clinical trial (NCT01522820), titled “A phase I clinical trial of mTOR inhibition with rapamycin for enhancing intranodal dendritic cell vaccine induced anti-tumor immunity in patients with NY-ESO-1 expressing solid tumors”.

2 OBJECTIVES

2.1 Phase I Primary Objectives

- To determine the safety of fixed doses of DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC given as a vaccine in combination with INCB024360.
- To evaluate toxicity as defined by NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

2.2 Phase IIb Primary Objectives

- To determine the progression free survival (PFS) (primary endpoint) using standard irRC criteria.

2.3 Secondary Objectives (Phase I, Phase IIb and Exploratory Cohort)

- To determine the effectiveness of INCB024360 on enhancing vaccine efficacy by assessing NY-ESO-1 specific cellular and humoral immunity.
- Exploratory Cohort at Roswell Park ONLY: To determine the effectiveness of Sirolimus on enhancing vaccine efficacy by assessing NY-ESO-1 specific cellular and humoral immunity.
    - Peripheral blood NY-ESO-1 specific CD8+ and CD4+ T cells.
    - Peripheral blood NY-ESO-1 specific antibodies.
    - Peripheral blood frequency of CD4+CD25+FOXP3+ regulatory T cells.
    - Pharmacokinetics of INCB02360 in relation to T cell frequency and function in correlation with PFS.

3 METHODOLOGY

3.1 Study Design

This is an open-label, Phase I/IIb study of the IDO inhibitor, INCB024360, in combination with DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC in patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in remission.

For the Phase I component of the study, eligible patients will receive a fixed intracutaneous dose of DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with subcutaneous adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles; and Cohort 1 patient enrollment will begin INCB024360 at a fixed daily dose of 300 mg BID orally (Cohort 1) for a total of 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -1. Cohort -1 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 100 mg BID (Cohort -1) starting on Day 1 for 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -2. Cohort -2 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 50 mg BID (Cohort -1) starting on Day 1 for 7 cycles.

After completing 1 cycle of treatment in the last patient from Cohort 1 (or Cohort -1 or Cohort -2), accrual into the randomized Phase IIb study (Cohort 2) will begin at the dose of INCB024360 that is determined to be safe in the Phase I trial (300 mg BID versus 100 mg BID versus 50 mg BID) in combination with CDX-1401. This is a randomized parallel design employing randomization for each patient enrolled to 1 of 2 groups (**Table 1**). Treatment cohorts include (i) DEC205mAb-NY-ESO-01 fusion protein (CDX-1401) given with adjuvant poly-ICLC alone and (ii) INCB024360 plus DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC.

An additional exploratory cohort (N=6) receiving 4 cycles of sirolimus in combination with DEC205mAb-NY-ESO-01 fusion protein (CDX-1401) with adjuvant poly-ICLC will be added to supplement the secondary objectives, which will aid in planning the next phase of the investigation. This will not affect the randomized component of the study as enrollment to the randomized Phase IIb at Roswell Park only will be suspended temporarily until the 6 patients are enrolled. Data will be included as described in Section 11.4 for immune response.

3.1.1 Target Accrual and Study Duration

The Phase I component of this study will enroll 3 -18 patients and 50 patients for the Phase IIb component. Six patients will be enrolled in the additional exploratory cohort. Patients will be enrolled at Roswell Park and participating sites. The exploratory cohort applies to the Roswell Park site only. Accrual is expected to take approximately 8 years. Patients in Phase I and Phase IIb are expected to participate in this study for approximately 28 weeks of active therapy. Patients in the exploratory cohort are expected to participate in this study for approximately 20 weeks of active therapy.

4 PATIENT SELECTION

4.1 Inclusion Criteria

To be included in this study, patients must meet the following criteria:

1. Eligible patients will be women with epithelial ovarian, fallopian tube, or primary peritoneal carcinoma after chemotherapy with no evidence of disease or minimal residual disease for primary or recurrent disease. This may or may not be measurable. These patients would normally enter a period of observation after standard management.
2. Any HLA type. (Historic HLA typing is permitted.)
3. Tumor expression of NY-ESO-1 or LAGE-1 by IHC and/or RTPCR.
4. Life expectancy > 6 months.
5. Hematology and biochemistry laboratory results within the limits normally expected for the patient population, without evidence of major organ failure. Have the following clinical laboratory values:
   - ANC $\geq$ 1,000/μL
   - PLT $\geq$ 100,000/μL
   - Hgb $\geq$ 8 g/dL
   - Total bilirubin $\leq$ 1.5 x ULN
   - Serum aspartate aminotransferase (SGOT/AST) or serum alanine aminotransferase (SGPT/ALT) $\leq$ 3 x ULN
   - Serum creatinine $\leq$ 2 x ULN
6. Have been informed of other treatment options.

7. Age ≥ 18 years of age.
8. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.
9. Have an ECOG Performance Status of ≤ 2. Refer to **Appendix A**.
10. The ability to swallow and retain oral medication.
11. Patients of child-bearing potential must agree to use acceptable contraceptive methods (e.g., double barrier) during treatment.
12. Patients may have received previous NY-ESO-1 vaccine therapy. Patients who received maintenance paclitaxel or bevacizumab are eligible for enrollment provided they have discontinued therapy (at least 4 weeks for prior taxane or prior bevacizumab) prior to randomization and recovered from toxicities to less than Grade 2.

4.2 Exclusion Criteria

Patients will be excluded from this study for the following:

1. Metastatic disease to the central nervous system for which other therapeutic options, including radiotherapy, may be available.
2. Other serious illnesses (e.g., serious infections requiring antibiotics, bleeding disorders).
3. History of severe autoimmune disorders requiring use of steroids or other immunosuppressives.
4. Concomitant systemic treatment with chronic use (based on the investigator's judgment) of corticosteroids, anti-histamine or non-steroidal anti-inflammatory drugs, and other platelet inhibitory agents (See also **Section 6.4** for restrictions/recommendations on ancillary therapy).
5. Chemotherapy, radiation therapy, or immunotherapy within 4 weeks prior to first dosing of study drug (6 weeks for nitrosoureas). Concomitant hormonal therapies for breast cancers are allowed.
6. Subjects being treated with a monoamine oxidase inhibitor (MAOI), or drug which has significant MAOI activity (e.g., meperidine, linezolid, methylene blue) within 3 weeks prior to screening.
7. Subjects who are currently receiving therapy with a potent CYP3A4 inducer or inhibitor (e.g. clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir).
8. Use of UGT1A9 inhibitor including: diclofenac, imipramine, and ketoconazole.
9. Participation in any other clinical trial involving another investigational agent within 4 weeks prior to first dosing of study drug.
10. Known hepatitis B, hepatitis C, or HIV.

11. Mental impairment that may compromise the ability to give informed consent and comply with the requirements of the study.
12. Lack of availability of a patient for immunological and clinical follow-up assessment.
13. Evidence of current drug or alcohol abuse or psychiatric impairment, which in the Investigator's opinion will prevent completion of the protocol therapy or follow-up.
14. Pregnant or nursing female patients.
15. Unwilling or unable to follow protocol requirements.
16. Any condition which in the Investigator's opinion deems the patient an unsuitable candidate to receive study drug (i.e., any significant medical illness or abnormal laboratory finding that would, in the investigator's judgment, increase the patient's risk by participating in this study).
17. Known hypersensitivity to any of the study drugs that will be given to the participant
18. Additional exclusion criteria for exploratory cohort ONLY: Known pulmonary hypertension.

4.3 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this study.

5 INVESTIGATIONAL AGENTS

5.1 DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401)

Detailed technical information regarding CDX-1401 can be found in the Investigator's Brochure. CDX-1401 is a fusion protein consisting of a fully human monoclonal antibody (HuMab) of IgG1 (kappa) isotype with specificity for the dendritic cell receptor, DEC-205, genetically linked to the full length NY-ESO-1 tumor antigen.

The clinical trial product will be formulated as a sterile solution intended for parenteral use. CDX-1401 will be provided in 2 mL vials containing approximately 1 mL of solution, at a concentration of 1 mg/mL. CDX-1401 vials should be stored at -20˚C ± 5˚C the in pharmacy department.

Source: Celldex Therapeutics, Inc, Fall River, MA.

Intended Dose: 1 mg on Day 1 of each cycle.

5.2 Poly-ICLC Adjuvant

Poly-ICLC (polyinosinic-polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose) is a synthetic double-stranded ribonucleic acid (dsRNA) with broad immune-enhancing effects mediated through TLR-3 activation.

Poly-ICLC will be provided in single-dose vials containing 1 mL of opalescent solution. Poly-ICLC drug product used to be labeled as 2 mg/mL but as per IB V14 (1/25/2018), this is being changed to 1.8 mg/mL to incorporate for the water content in the lyophilized components (Poly I and Poly C). This represents no change in the product or formulation, but simply a calculation correction and therefore, irrespective of being labeled differently (older lots 2.0 mg/mL label and newer lots 1.8 mg/mL label) dosing volume for this study remains unchanged. In effect, the prior 2.0 mg/mL label represented the "wet" Poly-IC concentration which is equivalent to the current 1.8 mg/mL label representing the "dry" Poly-IC concentration. The current lot labeled 2.0 mg/mL will expire on 2/28/18 and pharmacy will start using the new lot with the new labels (1.8 mg/ml) effective 3/1/18.

The poly-ICLC vials should be refrigerated at about 40ºF (2 ºC - 8ºC) but should not be frozen. Poly-ICLC is also stable at room temperature for brief periods (days).

Because the product is not formulated with a preservative, once the sterile vials are entered or the poly-ICLC is drawn into a syringe, the drug should be used as soon as possible (typically within 6 hours if kept at room temperature or within 24 hours if refrigerated and the syringe(s) had been filled under aseptic conditions) or in accordance with any applicable institutional guidance. Poly-ICLC is to be withdrawn from the vial under sterile conditions and administered subcutaneously as supplied.

Source: Poly-ICLC is an Investigational Product manufactured by Oncovir for Dalton, Washington, DC, USA; will be supplied by Celldex Therapeutics, Inc, Fall River, MA.

Intended Dose: 1 ml on Day 1 and Day 2 of each cycle.

All synthesis, production, formulation, and packaging of the investigational agents are in accordance with applicable current Good Manufacturing Practices and meet applicable criteria for use in humans.

5.3 INCB024360

INCB024360 drug substance is a white to off white powder and is referred to herein as INCB024360. INCB024360 drug product will be provided as 25 mg, 100 mg, and 300 mg tablets. The tablet formulation contains the active ingredient along with commonly used excipients. The tablets contain the active drug (INCB024360) along with commonly used excipients Lactose Monohydrate, Microcrystalline Cellulose, Povidone, Croscarmellose Sodium Colloidal Silicon Dioxide, and Magnesium Stearate. All excipients are of compendial grade.

Source: Incyte Corporation Wilmington, DE 19880

Intended Dose: 50 mg, 100 mg, and 300 mg BID, depending on the cohort.

5.4 Sirolimus (exploratory cohort at Roswell Park only)

5.4.1 Active Substance and Source

Each tablet of sirolimus contains either 1 mg or 2 mg. Sirolimus will be purchased by Roswell Park Investigational Drug Services with grant funding.

5.4.2 Packaging and Labeling

Either generic or brand name Sirolimus can be used in this study at a dose of 4 mg/day.

5.5 Storage and Stability

The Investigator or designate is responsible to store and dispense the investigational product and will be responsible for ensuring that study drug is securely maintained in a locked, limited-access facility, in accordance with the applicable regulatory requirements. Drug storage temperature will be maintained, recorded, dated, and initialed daily, as applicable.

Sirolimus is chemically stable at room temperature.

5.6 Handling and Disposal of Investigational Products

The Investigator or designate will be responsible for dispensing and accounting for all investigational drug provided by Roswell Park exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken per the institution's environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

Study drug accountability records will be available for verification by the Clinical Research Monitor appointed from the CRS office at each monitoring visit. At the completion of the study, there will be a final reconciliation of all study drug.

An investigational agent dispensing record will be kept current and will contain the following information:

- Patient's identification information (i.e., patient number and initials).
- Date and quantity of drug dispensed.
- Date and quantity of drug returned to the Investigator/pharmacy (if any).
- Date and quantity of accidental loss of study drug (if any).

These inventories must be made available for inspection by the CRS study monitor. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

6 TREATMENT PLAN

6.1 Dosing and Administration

Treatment will be administered on an outpatient basis.

For the Phase I component of the study, eligible patients will receive a fixed intracutaneous dose of DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with subcutaneous adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles; and Cohort 1

patient enrollment will begin INCB024360 at a fixed daily dose of 300 mg BID orally (Cohort 1) for a total of 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -1. Cohort -1 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 100 mg BID (Cohort -1) starting on Day 1 for 7 cycles. Based on the decision rules for DLTs, patients may be enrolled into Cohort -2. Cohort -2 will receive DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) (Day 1) given with adjuvant poly-ICLC (Day 1 and Day 2) for each 28 day cycle for a total of 5 cycles, and daily oral INCB024360 50 mg BID (Cohort -1) starting on Day 1 for 7 cycles.

After completing 1 cycle of treatment in the last patient from Cohort 1 (or Cohort -1 or Cohort -2), accrual into the randomized Phase IIb study (Cohort 2) will begin at the dose of INCB024360 that is determined to be safe in the Phase I trial (300 mg BID versus 100 mg BID versus 50 mg BID) in combination with CDX-1401. This is a randomized parallel design employing randomization for each patient enrolled to 1 of 2 groups. Treatment cohorts include (i) DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC alone and (ii) INCB024360 plus DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC.

An additional exploratory cohort (N=6) receiving 4 cycles of sirolimus in combination with DEC205mAb-NY-ESO-01 fusion protein (CDX-1401) with adjuvant poly-ICLC will be added to supplement the secondary objectives, which will aid in planning the next phase of the investigation. This will not affect the randomized component of the study as enrollment to the randomized Phase IIb at Roswell Park only will be suspended temporarily until the 6 patients are enrolled. Data will be included as described in Section 11.4 for immune response.

6.1.1 CDX-1401

CDX-1401 will be administered as a total dose of 1 mg based on the safety and immunogenicity assessment in the Phase I study of CDX-1401. CDX-1401 will be supplied at a concentration of 1 mg/mL. A 1 mL volume is to be administered and should be split into 2 intracutaneous injections given within the 5 cm x 5 cm administration site.

Intracutaneous injection is a combination of intradermal and subcutaneous administration. The maximum volume which can be administered intradermally is 0.1 ml and the maximum volume administered subcutaneously is considered to be 1-2 ml. Each injection will be given intracutaneously (0.1 ml intradermally and the remainder subcutaneously) within the identified 5 cm X 5 cm treatment site.

Intracutaneous injection should be performed as follows: Small gauge needles (5/8 inch-1 inch by 25 gauge or smaller) are recommended for intracutaneous injections. A region of skin including the 5 cm x 5 cm administration site should be cleaned with an alcohol prep pad and allowed to air dry. Each of the 2 injections should consist of a combination of intradermal and subcutaneous administration. Beginning with the intradermal administration, the needle is advanced at least 1 cm into the dermis at a shallow angle, ensuring that the needle tip remains within the dermis. The injection should then begin; assuring that a skin bleb begins to form. If a bleb is not formed, the needle should be repositioned within the skin without being withdrawn, and the injection can be attempted again. Approximately 0.1 ml of each planned injection should be injected intradermally.

The needle is then backed away so that the tip is outside the bleb but still within the skin. It can then be advanced at a sharper angle to reach the superficial subcutaneous space underneath the bleb at a depth of approximately 5 mm. Provided no blood is aspirated, the remaining planned injection volume is administered. This procedure is repeated for the remaining injection until the total planned volume of 1 mL is administered.

The administration site should be free of potentially complicating dermatologic conditions, and should not be located in an area where integrity of the draining lymph node bed is potentially compromised (i.e., an extremity where a nodal resection was previously performed). For each subsequent administration cycle, a new administration site should be selected, with the aim of stimulating a new draining lymph node bed for each administration. All nurses administering this vaccine will be trained to administer the doses intracutaneously prior to study implementation.

6.1.2 Poly-ICLC

Poly-ICLC dosing will consist of 1 subcutaneous injection of 1 mL administered by the clinic personnel within 5x5 cm area of CDX-1401. Adjuvant poly-ICLC will be administered as soon as possible after each CDX-1401 injection, with the goal to administer the vaccine and adjuvant within a 1 hour period. The second poly-ICLC administration for each cycle may be self-administered by the patient 24 hours (± 6 hours) later for Cycle 2 onward. Patients will be properly educated and instructed regarding the storage of poly-ICLC; when, how, and where to self-administer the product; and to maintain the patient diary. (**Appendix C**).

6.1.3 INCB024360

INCB024360 is administered at varying dose levels depending on the cohort a patient is assigned (**Table 1**). Patients will be instructed on the dose and schedule of INCB024360 for the cohort to which they have been assigned. Tablets will be taken in the morning and evening, approximately 12 hours apart and at least 2 hours after a meal. Patients will abstain from food for 1 hour after dosing. Each cycle is a period of 28 days.

6.1.4 Sirolimus (Exploratory cohort at Roswell Park ONLY)

Sirolimus is administered to patients enrolled in the exploratory cohort at Roswell Park ONLY. A fixed dose of Sirolimus 4mg/day for the first 2 weeks of each 28 day cycle will be given for four cycles as described in Table 2 and Table 4. Sirolimus will not be taken with the 5th vaccine.

6.1.5 Compliance

CDX-1401 and poly-ICLC will be administered by the study staff on the CDX-1401 dosing dates, and may be self-administered by the patient for the remaining doses. Compliance with self-administration of adjuvants and INCB024360 (or Sirolimus for exploratory cohort) will be assessed via patient-reported dosing diaries (**Appendix C** and **Appendix D**).

Table 1. Dosing Schedule for INCB024360 and DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC

Treatment Cohorts	Number of Patients	DEC205mAb-NY ESO 1 Fusion Protein (CDX 1401) Given with Adjuvant Poly-ICLC[a]	INCB024360 (Oral)
Phase I			
Cohort -2	3 – 6 patients	• DEC205mAb-NY-ESO-1 fusion protein (1 mg, intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles. • Adjuvant poly-ICLC (1 ml, subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles.	50 mg BID for a total of 7 cycles
Cohort -1	3 – 6 patients	• DEC205mAb-NY-ESO-1 fusion protein (1 mg, intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles. • Adjuvant poly-ICLC (1 ml, subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles.	100 mg BID for a total of 7 cycles
Cohort 1 (start)	3 – 6 patients	• DEC205mAb-NY-ESO-1 fusion protein (1 mg, intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles. • Adjuvant poly-ICLC (1 ml, subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles.	300 mg BID for a total of 7 cycles
Randomized Phase IIb (parallel groups design)			
Cohort 2a	25 patients	•DEC205mAb-NY-ESO-1 fusion protein (1 mg. intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles Adjuvant poly-ICLC (1 ml. subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles..	None
Cohort 2b	25 patients	• DEC205mAb-NY-ESO-1 fusion protein (1 mg, intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles. • Adjuvant poly-ICLC (1 ml, subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles.	300 mg BID for a total of 7 cycles

1 Refer to **Section 11** statistical considerations and additional decision rules.

Based on phase 1 safety review, INCB024360 300 mg. po BID for a total of 7 cycles was determined the phase IIb dose level.

Table 2 Exploratory Cohort Dosing Schedule for Sirolimus and DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC

NOTE: Exploratory Cohort enrollment at Roswell Park site ONLY.

Treatment Cohorts	Number of Patients	DEC205mAb-NY ESO 1 Fusion Protein (CDX 1401) Given with Adjuvant Poly-ICLC[a]	Sirolimus
Exploratory Cohort			
Cohort 3	6 patients	• DEC205mAb-NY-ESO-1 fusion protein (1 mg, intracutaneous injection) on Day 1 of each 28 day cycle for a total of 5 cycles. • Adjuvant poly-ICLC (1 ml, subcutaneous) on Day 1 and Day 2 of each 28 day cycle for a total of 5 cycles.	4 cycles of 4 mg/day for 2 weeks followed by 2 weeks off

6.2 Cohort Management

A patient will be considered to have completed treatment with study medications following completion of 7 cycles of treatment and the final assessments at treatment discontinuation. An eligible patient receiving at least 1 dose of the investigational agents, DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC with or without INCB024360, will be considered evaluable for safety. A patient that completes 2 or more cycles of treatment will be eligible for the analysis of immune response. Patients who do not complete the study through Day 29 for reasons other than dose limiting toxicities (DLTs) will be replaced. All patients will be included in the analysis of results.

6.3 Dose Modification

No dose adjustments during this study are planned for the DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC vaccine.

In the event of excessive discomfort, skin breakdown or desquamation, or a Grade 3 treatment-related systemic toxicity following CDX-1401 and poly-ICLC dosing, administration of adjuvant (poly-ICLC) on the subsequent day should be omitted. Provided the toxicity has resolved to Grade 2 or less, the next monthly treatment may be administered as scheduled at a new administration site.

Refer to **Section 11.1** for the Phase I parameters and decisions rules for INCB024360 dose adjustments.

Dose adjustments for hematological toxicity will be based on the blood counts obtained at baseline and after each 28-day cycle of DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC and with or without INCB024360.

If at least 2/3 or 2/6 DLT's are observed in cohort one, the dose of INCB024360 will be reduced from 300 mg to 100 mg BID. If at least 2/3 or 2/6 DLT's are observed in Cohort 1, the dose of INCB024360 will be reduced from 100 mg to 50 mg BID in Cohort 2. The study will continue at this dose level.

Dose modification for INCB024360 are based on decision rules in **Section 11.1**.

Patients will be withdrawn from the study if they fail to recover to Grade 2, CTCAE Version 4.0, from a treatment-related toxicity within 4 weeks (leading to treatment delay of ≥ 4 weeks. For patients that recover in < 4 weeks, when toxicities have resolved to Grade 2 or lower, INCB024360 may be restarted. In patients that recover from toxicity in less than 4 weeks and who restart therapy, the next treatment cycle will be scheduled for 4 weeks later. Additional cycles will also follow at 4 weeks intervals.

Dose modification for Phase IIB (INCBO24360 cohorts)

When an INCBO24360 toxicity is observed, dose administration may be delayed/interrupted to allow for recovery from toxicity. INCB024360 may be restarted at a reduced dose of 100 mg. po BID. Please contact the PI for attribution.

Based on the phase I safety review, INCBO24360 300 mg. po BID x 7 cycles has been selected for the phase IIB dose. Dose modification criteria will be defined as any Grade 3 or higher treatment-related (i.e. definitely, probably, or possibly related) toxicity that occurs outside the DLT evaluation period including:

- Febrile neutropenia with standard of care treatment for ≥ 7 days. Growth factors may be used according to American Society of Clinical Oncology guidelines.
- Platelets ≤ 50 x 10^9/L
- Grade 3 or greater non-hematologic toxicity
- Serum bilirubin > 3.0 mg/dL
- AST / ALT > 3.5 x ULN
    - AST normal range: 15 U/L – 46 U/L
    - ALT normal range: 11 U/L - 66 U/L
- Serum creatinine ≥ grade 3 (equal or greater) will be considered a DLT
- Grade 3 or greater hypersensitivity reaction. If the hypersensitivity reaction is determined to be related to INCB024360 alone, the INCB024360 will be discontinued and patient may continue on treatment with CDX-1401 with Poly-ICLC at the discretion of the investigator.

Dose modification for exploratory cohort with Sirolimus (Roswell site ONLY):

Based on the completed trial (I 191511 "A Phase 1 Clinical Trial of mTOR Inhibition With Rapamycin for Enhancing Intranodal Dendritic Cell Vaccine Induced anti-Tumor Immunity In Patients with NY-ESO-1 Expressing Solid Tumors"), the 4 mg/day Sirolimus dose was safely used in combination with DEC205 and therefore dose modification is not expected. However, in the event of an unexpected toxicity attributed to Sirolimus, a lower dose of Sirolimus may be considered.

6.3.1 Definition of Dose-Limiting Toxicity

Patients who do not complete the study through Day 29 for reasons other than dose limiting toxicities (DLTs) will be replaced. All patients will be included in the analysis results.

All AEs (as defined in **Section 9**) will be graded according to the NCI-CTCAE scale Version 4.0. Patients who experience a DLT will not receive any additional treatment (i.e., will be removed from the study and followed until resolution of their toxicity) and will not receive continued treatment even at a reduced dosage of the INCB024360 product. Patients with dose limiting toxicities requiring drug discontinuation (specified below) will be removed from the study (see exception for hypersensitivity reaction). The DLT period will be defined as 28 days after the first dose of vaccine. Late developing toxicities, occurring between Day 29 and Day 183 will be monitored throughout the study and according to the schedule of procedures and observations. Patients may be withdrawn from study even if DLT is due to INCB024360 or sirolimus alone. Safety monitoring will include careful assessment and appropriate reporting of AEs, as well as the construction and implementation of a data and safety-monitoring plan (Auditing, Monitoring, and Inspecting). Medical monitoring will include a regular assessment of the number and type of SAEs. Refer to the stopping rules for this study in **Section 6.3.2**. Any event(s) that might appear to trigger a treatment discontinuation rule or trial stopping rule will be discussed with the FDA review team. For phase IIb dose reductions for INCB024360 will be allowed (see section 6.3)

Management and dose modifications associated with AEs are outlined in **Section 6.3**. Refer to **Section 11** for statistical considerations and additional decision rules.

These events should be deemed to be possibly, probably or definitely related to study drugs. DLT criteria will be defined as any Grade 3 or higher treatment-related (i.e. definitely, probably, or possibly related) toxicity that occurs during the DLT evaluation period, including:

- Febrile neutropenia with standard of care treatment for ≥ 7 days. Growth factors may be used according to American Society of Clinical Oncology guidelines.
- Platelets ≤ 50 x 10^9/L
- Grade 3 or greater non-hematologic toxicity
- New onset pulmonary hypertension, interstitial pneumonitis or, CNS bleeding
- Serum bilirubin > 3.0 mg/dL
- AST / ALT > 3.5 x ULN
    - AST normal range: 15 U/L – 46 U/L
    - ALT normal range: 11 U/L - 66 U/L
- Serum creatinine ≥ grade 3 (equal or greater) will be considered a DLT
- Grade 3 or greater hypersensitivity reaction. If the hypersensitivity reaction is determined to be related to INCB024360 alone, the INCB024360 will be discontinued and patient may continue on treatment with CDX-1401 with Poly-ICLC at the discretion of the investigator.

6.3.2 Management of Delayed Events

Late-developing toxicities, occurring between Day 29 and Day 143 will be monitored throughout the study and according to the schedule of procedures and observations.

Study Stopping Rules

Any Grade 4 event will trigger a study pause for data review prior to further enrollment. In addition, the following stopping rule for treatment-related SAEs will be used:

- Accrual to study will be suspended to allow protocol review with institutional IRB, the Early Phase Clinical Trials (EPCT) Committee, and the FDA review team if any of the following occurs among patients who receive experimental agents:
    - Any treatment-related death, judged to be possibly, probably, or definitely related to INCB024360 or DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC.
    - Exploratory cohort at Roswell Park ONLY: Any treatment-related death, judged to be possibly, probably, or definitely related to sirolimus or DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant poly-ICLC.
    - Grade 4 events considered to be possibly, probably, or definitely related to the experimental agents.

6.4 Ancillary Therapy and Supportive Care

Patients may not receive chronic treatment during the period of study with systemic corticosteroids or other immunosuppressive agents including chemotherapeutic agents. A brief course of systemic corticosteroids can be used to manage immune based toxicities, if deemed appropriate by the treating physician. Treatment with NSAIDs should be avoided during the study. Investigators may prescribe all other concomitant medications or treatments deemed necessary to provide adequate patient care.

Concurrent non-cytotoxic anti-cancer therapy (e.g., hormone therapy) is permitted.

The electronic case report form (eCRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies, listing drug name, indication, dose, route of administration, and dates of administration.

Patients may be pretreated for nausea and vomiting with appropriate anti-emetics.

6.4.1 Permitted Treatment

- Any medication for concurrent medical condition.
- Inhaled corticosteroids, if indicated.
- If clinically indicated, use of growth factors according to American Society of Clinical Oncology guidelines.
- Antiemetics for breakthrough nausea and vomiting:

- Metoclopramide 1 mg - 2 mg/kg IV every 3 to 4 hours p.r.n. nausea/vomiting.
- Prochlorperazine 10 mg IV/PO every 4 to 6 hours p.r.n. nausea/vomiting.
- Lorazepam 0.5 mg - 2 mg PO/SL/IV every 4 to 6 hours p.r.n nausea/vomiting.
- Olanzepine 2.5 mg - 5 mg PO twice daily.

- Use of coumarin-based anticoagulants (e.g. Coumadin) is discouraged. Low-dose coumadin (1 mg) is acceptable; however, doses that increase the INR are discouraged and will require dose modification. If an alternative to coumarin-based anticoagulants cannot be used, dose adjustment of the Coumadin may be needed. Based on the observed magnitude of epacadostat/warfarin PK interaction and PK/PD modeling results, for an epacadostat dose of 300 mg BID, the dose of warfarin should be reduced by approximately one-third after initiation of epacadostat administration based on approximately 30% to 40% reduction in S- and R-warfarin oral clearance values. Close INR monitoring is recommended for patients on a stable dose of warfarin who are starting treatment with epacadostat. Based on PK/PD modeling, recommendations for warfarin dose modifications for subjects receiving other epacadostat doses, are summarized below based on the INR prior to starting epacadostat.

Stable Baseline INR	**Epacadostat Dose**		
	≤ 100 mg BID	**200 mg BID**	**300 mg BID**
INR ≤2.5	Close INR monitoring	Close INR monitoring	Reduce warfarin by~33% and monitor INR
INR >2.5	Close INR monitoring	Reduce warfarin by 20-25%/monitor INR	Reduce warfarin by ~33%/ monitor INR

6.5 Prohibited Treatment

Chemotherapy, radiation, surgery, immunosuppressive therapies are prohibited throughout this study. Patients being treated with a monoamine oxidase inhibitor (MAOI), or drug which has significant MAOI activity (e.g., meperidine, linezolid, methylene blue) within 3 weeks prior to screening is prohibited. Patients who are currently receiving therapy with a potent CYP3A4 inducer or inhibitor is prohibited. Use of UGT1A9 inhibitor including: diclofenac, imipramine, and ketoconazole is prohibited. Inhaled corticosteroids or short course of systemic corticosteroids are permitted. Daily nonsteroidal anti-inflammatory drugs for more than 2 weeks are not permitted.

Sirolimus Exploratory Cohort: At the investigator's discretion full dose anti-coagulation therapy may need to be adjusted for study procedures. Daily nonsteroidal anti-inflammatory drugs for more than 2 weeks are not permitted. The use of posaconazole and voriconazole are contraindicated because of drug-drug interactions. An additional list of drugs that have theoretical or probable interaction with sirolimus is provided in Appendix B**.** Patients taking any of these drugs will be

monitored clinically. Treatment with corticosteroids should be avoided during this study. However, a short duration (≤ 2 weeks) with systemic corticosteroids will be allowed for treatment of acute events.

6.6 Duration of Treatment

The duration of patient enrollment is expected to be 8 years.

INCB groups: Patients are expected to participate in the study for approximately 28 weeks of active therapy and follow-up assessments at 3 months, 6 months, and 12 months after the last dose of study drug. Patients may return to standard of care after completing all assessments at follow-up.

Exploratory Cohort with Sirolimus: Patients are expected to participate in the study for approximately 20 weeks of active vaccine therapy and follow-up assessments at 3 months, 6 months and 12 months after the last dose of study drug. Patients may return to standard of care after completing all assessments at End of Treatment.

6.7 Treatment Discontinuation

Upon treatment discontinuation all end of study evaluations and tests will be conducted. All patients who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the patient's medical records and the appropriate eCRF. If patient terminates study participation prior to study completion, all efforts will be made to complete an early termination visit (within approximately 30 days of last dose). Assessment will include physical exam, vital signs and weight, performance status, labs (CBC/diff, CMP, LDH, Coag if on anticoagulants, con med/AE assessment.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Treatment-related toxicity
    - Subjects who experience a DLT will not receive any additional treatment (i.e., will be removed from the study and followed until resolution of their toxicity) and will not receive continued treatment even at a reduced dosage of the INCB024360 product.
    - Persistent toxicity that is unresolved to a grade 2 toxicity, where subsequent treatment cannot be given.
- Toxicity unrelated to treatment
- Investigator judgment

  - The Investigator may withdraw a patient if, in his/her judgment, it is in the patient's best interest to do so.

- Noncompliance
- Patient voluntary withdrawal
  - A patient may withdraw from the study at any time, for any reason. If a patient discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Roswell Park's decision.

Patients who are unavailable for follow-up evaluations should be classified as lost to follow-up for 1 of the following reasons:

- Lost to follow-up: For a patient to be considered lost to follow-up, the Investigator must make 2 attempts to re-establish contact with the patient. The attempts to re-establish patient contact must be documented (e.g., certified letter).
- Death: Date and cause of death will be recorded for those patients who die within 30 days after last dose of study drug (telephone contact is acceptable).

7 STUDY PROCEDURES

Eligibility of each patient will be established prior to enrollment. Informed consent ***MUST*** be completed prior to receiving any study related procedures. Details regarding the schedule of procedures and observations are outlined below and in **Table 3**.

7.1 Vaccination Site Care

After the administration of the vaccine to the patient, the vaccination site will be covered loosely with a gauze bandage, using first aid adhesive tape to keep it in place.

The patient will be instructed as follows:

- Keep the vaccination site dry and cover the vaccination site with a waterproof bandage when bathing.
- Change bandage to the loose gauze bandage after bathing.
- **DO NOT** use a bandage that blocks all air from the vaccination site. This may cause the skin at the vaccination site to soften and wear away. Use loose gauze secured with medical tape to cover the site.
- **DO NOT** put salves or ointments on the vaccination site.

7.2 Unscheduled Visit

The Investigator may, at his/her discretion, arrange for a patient to have an unscheduled visit, especially in the case of AEs that require follow-up. If a patient is experiencing an AE considered by the Investigator to be possibly related to the study drug, an unscheduled visit should be performed; the unscheduled visit pages for the eCRF must be completed.

7.3 Schedule of Procedures and Observations

Clinical assessments will be performed every 4 weeks. Imaging with computed tomography (CT) or magnetic resonance imaging (MRI) will be performed according to standard of care. Performance status and AEs will be assessed at each clinical assessment until disease progression.

The schedule of procedures and observations for the INCB024360 cohorts in this study is summarized in **Table 3** below. Approximately ± 3 days are allowed for the schedule of procedures.

The schedule of procedures and observations for the exploratory cohort (with sirolimus) at Roswell Park site ONLY is summarized in Table 4.

Table 3. Schedule of Procedures and Observations (INCB024360 cohorts)

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	Cycle 7	Treatment discontinuation[13]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of INCB
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 1 ± 3 days	[11]	
Informed Consent	X															
Medical History	X	X			X		X		X		X		X	X	X	
Physical Examination, vital signs and weight	X	X			X		X		X		X		X	X	X	
NY-ESO-1, TIL, PD-L1, and/or LAGE-1 Expression in Tumor Tissue[2]	X															
HLA Typing (Historic HLA Typing is Permitted-low resolution)[3]	X															
Disease Assessment[4]	X								X						X	X[5]
Hematology, PT/INR[6]	X				X		X		X		X		X	X	X	
Chemistry[7]	X				X		X		X		X		X	X	X	
Pharmacokinetic/Pharmacodynamic Sampling[8]		X		X	X											
Performance Status (Appendix A)	X	X			X		X		X		X		X	X	X	
Tumor Markers[9]	X	X			X		X		X		X		X	X	X	
Vaccination: DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) given with Adjuvant Poly-ICLC		X			X		X		X		X					

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	Cycle 7	Treatment discontinuation[13]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of INCB
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 1 ± 3 days	11.1	
Adjuvant Poly-ICLC (administered in clinic or at home by the patient)		X	X		X	X	X	X	X	X	X	X				
INCB024360[10]		X	X	X	X	X	X	X	X	X	X	X	X	X		
Antibody Response: NY-ESO-1 ELISA and NY-ESO-1 Cellular Immune Response (T cell): ELISPOT and ICS[11]		X			X		X		X		X				X	X
Urinary Pregnancy Test in Females of Childbearing Potential	X															
Concomitant Medications	X[12]	X			X		X		X		X		X	X	X	
Adverse Events	X	X			X		X		X		X		X	X	X	

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	Cycle 7	Treatment discontinuation[13]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of INCB
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 1 ± 3 days	.1.1	

1 To be performed within 28 days prior to the first vaccination.

2 Positive by either PCR or immunohistochemistry ((archived tissue (**Appendix F**) and/or frozen/fresh tumor tissue (**Appendix G**)). Documented historical NY ESO 1 positive results by IHC **or** PCR **or serum antibody reactive with ESO** are acceptable.

3 Refer to **Appendix H**

4 Including CT scan abdomen and pelvis, or PET/CT as described in **Section 8.5**; within 28 days prior to first vaccination. If patient early terms, CT will be performed at the PI's discretion.

5 Only 3 months and 6 month for those patients who have not experienced disease progression.

6 Hematology includes: CBC with auto differential, platelets. PT/INR for patients on anticoagulants.

7 Chemistry includes: glucose, urea nitrogen, creatinine, calcium, protein, albumin, bilirubin, AST, ALT, LDH, alkaline phosphatase.

8 Pharmacokinetic sampling is dependent upon cohort. Refer to **Section 7.7** for details.

9 Tumor markers to be obtained as per standard of care.

10 INCB024360 will be administered daily for 7 cycles.

11 Refer to **Section 7.5**.

12 Medications ongoing within 1 week prior to first dose of study drug.

13 Approximately 30 days after last.study drug, con meds and AE's will be followed. Telephone contact is allowed.

Table 4 Schedule of Procedures/Observations (Exploratory cohort with Sirolimus-Roswell site ONLY)

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Treatment discontinuation[1,2]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of Sirolimus
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2		
Informed Consent	X													
Medical History	X	X		X	X		X		X		X		X	
Physical Examination, vital signs and weight	X	X		X	X		X		X		X		X	
NY-ESO-1, TIL, PD-L1, and/or LAGE-1 Expression in Tumor Tissue[2]	X													
HLA Typing (Historic HLA Typing is Permitted-low resolution)[3]	X													
Disease Assessment[4]	X								X				X	X[5]
Hematology, PT/INR[6]	X	X		X	X		X		X		X		X	
Chemistry[7]	X	X		X	X		X		X		X		X	
Performance Status (Appendix A)	X	X			X		X		X		X		X	
Tumor Markers[8]	X	X			X		X		X		X		X	

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Treatment discontinuation[1,2,12]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of Sirolimus
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2		
Vaccination: DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) given with Adjuvant Poly-ICLC		X			X		X		X		X			
Adjuvant Poly-ICLC (administered in clinic or at home by the patient)		X	X		X	X	X	X	X	X	X	X		
Sirolimus[9]		X	X		X	X	X	X	X	X				
Antibody Response: NY-ESO-1 ELISA and NY-ESO-1 Cellular Immune Response (T cell): ELISPOT and ICS[10]		X			X		X		X		X		X	X
Urinary Pregnancy Test in Females of Childbearing Potential	X													
CMV PCR, Qualitative	X								X				X	
Lipid profile[13]	X			X	X				X					
Concomitant Medications	X[11]	X		X	X		X		X		X		X	
Adverse Events	X	X		X	X		X		X		X		X	

Study Event	Baseline[1]	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Treatment discontinuation[12]	3 Months, 6 Months, and 12 Months After Last Vaccine or dose of Sirolimus
		Day 1	Day 2	Day 15	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2	Day 1 ± 3 days	Day 2		
.1,2														

1 To be performed within 28 days prior to the first vaccination.

2 Positive by either PCR or immunohistochemistry ((archived tissue (**Appendix F**) and/or frozen/fresh tumor tissue (**Appendix G**)). Documented historical NY ESO 1 positive results by IHC **or** PCR **or serum antibody reactive with ESO** are acceptable.

3 Refer to **Appendix H**

4 Including CT scan abdomen and pelvis, or PET/CT as described in **Section 8.5**; within 28 days prior to first vaccination. If patient early terms, CT will be performed at the PI's discretion.

5 Only 3 months and 6 month for those patients who have not experienced disease progression.

6 Hematology includes: CBC with auto differential, platelets. PT/INR for patients on anticoagulants.

7 Chemistry includes: glucose, urea nitrogen, creatinine, calcium, protein, albumin, bilirubin, AST, ALT, LDH, alkaline phosphatase.

8 Tumor markers to be obtained as per standard of care.

9 4 cycles of Sirolimus 4mg/day will be given for 2 weeks, followed by 2 weeks off (each cycle = 28 days).

10 Refer to **Section 7.5**.

11 Medications ongoing within 1 week prior to first dose of study drug.

12 Approximately 30 days after last study drug, Con meds and AE's will be followed. Telephone contact is allowed.

13 Patients known from clinical history to have elevation of lipids and triglycerides will be followed per standard guidelines. Lipid profile will be obtained as lipid panel including: Cholesterol, triglycerides, LDL, HDL.

7.4 Pathology

7.4.1 Baseline Tumor Tissue Requirements

Tumor tissue requirements for IDO, NY-ESO-1, TIL, PD-1, PD-L1 and/or LAGE-1, Expression in Tumor are as follows:

- Evaluation of NY-ESO-1 will occur at OmniSeq.
    - 5 unstained sections (5µm thick) and 8 tissue curls (10 µm thick) cut with molecular precautions.
- Evaluation of all other biomarkers will occur in the Pathology Network Shared Resource at Roswell Park.
    - 8 unstained sections (5µm thick) on plus glass
- Evaluation by PCR will occur in Immune Analysis Facility at the Center for Immunotherapy at Roswell Park. The director of the center is Junko Matsuzaki, Cancer Cell Center, Roswell Park, Room C-410, phone number 716-845-8459, e-mail address, junko.matsuzaki@roswellpark.org. Refer to **Appendix G**.
    - This will be performed when fresh/frozen tumor tissue is available.
- Documented historical NY ESO 1 positive results by IHC **or** PCR **or serum antibody reactive with ESO** are acceptable.

Network Site see Appendix J for specific instruction.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

7.4.2 Recurrence Tumor Tissues Requirements

The standard of care in patients who develop recurrent cancer, for the most part, is an additional cytotoxic chemotherapy. In order to ascertain whether treatment failure is a consequence of antigenic loss or represents failure of this strategy, it will be important to analyze tissue specimens from patients with recurrent disease. In a subset of patients, surgical resection of recurrent/progressive disease will be indicated as part of standard care. Specimens obtained at the time of surgery for recurrent/progressive disease will be analyzed for antigen expression and antigen-specific tumor infiltrating lymphocytes. The basic panel of markers noted in **Section 7.4.1** will also be tested. Up to 25 unstained sections of formalin-fixed paraffin-embedded recurrent tumors cut at 5 µm thickness on plus slides from the same population of patients will be used. The paraffin-embedded archival specimens will be obtained from the paraffin-archive resource in Roswell Park's Department of Pathology. Fresh or frozen tumor tissue will be obtained from Roswell Park Protocol I 115707 titled "Roswell Park Remnant Clinical Biospecimen Storage,

Collection, and Distribution for Research Purposes", or I 215512 titled "Analysis of Tumor Antigens, Immunity, and Genetic Changes in Gynecological Cancer Patients".

7.5 Immunological Response

INCB024360 Cohorts:

70 mL of blood for immunological analysis (humoral and cellular immunity) will be collected via venipuncture using 6 green top and 1 red top collection tube. Sample collection will be obtained at the following visits:

- Cycle 1 Day 1
- Cycle 2 Day 1
- Cycle 3 Day 1
- Cycle 4 Day 1
- Cycle 5 Day 1
- Treatment discontinuation
- 3 Months, 6 Months, and 12 Months After Last Vaccine or last dose of INCB

Assays of samples to determine immune responses will be performed in the Immune Analysis Facility at the Center for Immunotherapy at Roswell Park.

Network Site see Appendix H for specific instruction.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

Sirolimus Cohorts:

Blood samples will be collected via venipuncture for antibody response and cellular (T cell) immune response using ELISA for NY-ESO-1 and ELISPOT/ ICS (intracellular staining) respectively. Samples (70 ml) will be collected using 6 green top and 1 red top collection tube.

These samples for immunological analyses will be collected at the following visits:

- Cycle 1 Day 1
- Cycle 2 Day 1
- Cycle 3 Day 1
- Cycle 4 Day 1
- Cycle 5 Day 1

- Treatment discontinuation
- 3 Months, 6 Months, and 12 Months After Last Vaccine or last dose of sirolimus.

Samples will be kept at room temperature prior to processing. All samples to be sent to Immune Analysis Facility at Center for Immunotherapy:

Director: Dr. Junko Matsuzaki

CCC building, 4th floor

Phone 716-845-8459

Junko.Matsuzaki@RoswellPark.org

Note: All laboratories housing research samples need to maintain current, study-specific **Temperature Logs** and **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

7.5.1 Humoral Immunity

Specific antibody against NY-ESO-1 will be measured by ELISA (see Reference [7] for methodology).

7.5.2 Cellular Immunity

NY-ESO-1 specific CD4+ and CD8+ T cells will be measured by IFNγ release ELISPOT and Intracellular Cytokine Staining (ICS). Peripheral blood frequency of CD4+ CD25+ FOXP3+ regulatory T cells. Assays will be performed at the Immune Analysis Facility at the Center for Immunotherapy, Roswell Park, Buffalo, NY [42-44] for methodology). In order identify the DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC vaccination plus INCB024360 (or sirolimus for exploratory cohort) schedule that is associated with optimal biological activity, the following parameters will be determined (i) generation of effector and memory T cells with (ii) high avidity that are also (iii) resistant to T_{regs}.

7.6 Additional Exploration of the T cell response

The methods to analyze the T cell response to cancer antigens used in this study are well established. However, as T cell biology and immune regulation are very active fields of discovery, the opportunity exists to refine and extend the analysis of the T cell response. Therefore, although most of the blood drawn for the analysis of the immune response will be used for the assays listed above, a small part may be used to explore other aspects of the T cell response. In this regard, we will examine the evolution of memory $CD8^+$ cells in the different patient cohorts. Using multi-parameter flow cytometry we will determine $CD8^+$ cell subsets as follows: (i) central memory $CD45RA^-CCR7^+CD27^+$ cells, which have the ability to migrate to lymph nodes; (ii) early and intermediate effector memory $CD45RA^-CCR7^-CD27^+$ cells, with high proliferative capacity and low cytolytic activity; (iii) $CD45RA^-CCR7^-CD27^-$ cells, so-called late effector memory cells, with strong effector functions such as cytolytic capacity; and (iv) $CD45RA^+CCR7^-CD27^-$ cells, known

as fully differentiated effector cells, which retain those functional capacities but are stably non-replicative and apoptosis-resistant memory cells. On the basis of the distinct phenotypes of effector and memory $CD8^+$ T cells generated by DEC205mAb-NY-ESO-1 fusion protein with adjuvant poly-ICLC vaccine in the presence or absence of IDO inhibition, we will determine whether the transition from an effector to a memory cell phenotype is an abrupt or gradual event, and whether this transition would occur simultaneously for different markers at the primary and secondary responses. This work may also involve establishing T cell lines and clones in culture, and collaborations with Investigators outside of Roswell Park.

7.7 Pharmacokinetic/Pharmacodynamic Blood Sample Collection and Processing

7.7.1 INCB024360 Levels

Whole blood samples for pharmacokinetic analysis of INCB024360 levels will be collected via venipuncture using (1) 4 mL purple-top EDTA collection tube per time point.

INCB024360 pharmacokinetic sample collection will be obtained on:

- Cohort -2, Cohort -1, and Cohort 1
    - Cycle 1 Day 1 and Day 15: predose and 1, 2, 4, and 6 hours post dose of INCB024360
    - Cycle 2 Day 1: trough, prior to vaccination
- Cohorts 2b
    - Cycle 1 Day 15: predose and 2 hours post dose of INCB024360
    - Cycle 2 Day 1: trough, prior to vaccination

Plasma will be separated by centrifugation at 4°C from whole blood within 30 minutes following the extraction and aliquotted into (2) cryovials per time-point.

Network Site see Appendix H for specific instruction.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

Tryptophan and Kynurenine Levels:

Whole blood samples for pharmacokinetic analysis of tryptophan and kynurenine levels will be collected via venipuncture using (1) 4 mL purple-top EDTA collection tube per time point.

Tryptophan and kynurenine pharmacokinetic sample collection will be obtained on:

- Cohort -2, Cohort -1, and Cohort 1
    - Cycle 1 Day 1 and Day 15: predose, 1, 2, 4, and 6 hours post dose of INCB024360

- Cycle 2 Day 1: trough, prior to vaccination

- Cohorts 2b
  - Cycle 1 Day 15: predose and 2 hours post dose of INCB024360
  - Cycle 2 Day 1: trough, prior to vaccination

Plasma will be separated by centrifugation at 4°C from whole blood within 30 minutes following the extraction and aliquotted into (2) cryovials per time-point.

Network Site see Appendix H for specific instruction.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

Sample Processing:

The screw cap polypropylene cryogenic tube will be labeled with the participant's MR number(for Roswell Park participants), patient's initials, patient's study number, clinical study number, protocol time point, dose, and protocol day. The label for each patient's sample will be supplied by Roswell Park. The samples will be immediately frozen at - 70ºC or below until analyzed at the Roswell Park's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility:

Roswell Park Bioanalytics, Metabolomics & Pharmacokinetics Core Facility, Center for Genetics and Pharmacology, Room L1-140, I 248613, Elm & Carlton Streets, Buffalo, New York 14262, BMPKCore@Roswell Park.org.

For additional information regarding the handling of pharmacokinetic samples, please contact Roswell Park's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility laboratory at 716-845-3303 (Tel) or 716-845-1579 (Fax). Joshua Prey (Joshua.Prey@roswellpark.org); John Wilton (John.Wilton@roswellpark.org)

PK blood samples will be sent to procurement lab for processing and storage. After hours samples will be processed in Lab Medicine.

Network Site see Appendix H for specific instruction.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

7.7.2 Sirolimus Levels

Pharmacokinetic analysis of sirolimus levels will not be done for patients participating in the exploratory cohort. Based on the completed trial (I 191511 "A Phase 1 Clinical Trial of mTOR Inhibition With Rapamycin for Enhancing Intranodal Dendritic Cell Vaccine Induced anti-Tumor Immunity In Patients with NY-ESO-1 Expressing Solid Tumors"), the 4 mg/day Sirolimus dose was safely used in combination with DEC205.

8 EFFICACY EVALUATIONS

8.1 Objective Tumor Response

All protocol-defined imaging studies must be performed at the investigative site or sponsor-approved facility using protocol-defined parameters. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. RECIST 1.1 will be used to assess objective tumor response.

8.2 Target Lesions – PHASE 1

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size. Lesions with the longest diameter (short axis for lymph nodes) and are ≥ 10 mm (CT and MRI), ≥ 15 mm lymph nodes, > 20 mm CXR and are for accurate repetitive measurements (either by imaging techniques or clinically) will be chosen. A sum of the longest diameter (short axis for lymph nodes) of all target lesions will be calculated and reported as the baseline sum diameters. This will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

- **Complete Response (CR):** Disappearance of all target lesions. Any lymph nodes must have a reduction in short axis to < 10 mm. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Progressive Disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as references the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameter while on study. Patients having a documented response with no confirmation of the response will be listed with stable disease.

8.3 Non-Target Lesions

All other small lesions (longest diameter < 10 mm or lymph nodes ≥ 10 mm to < 15 mm short axis) and non-measurable lesions (i.e., leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, blastic bone lesions, or abdominal masses / abdominal organomegaly identified by physical exam that is not measurable by imaging) should be identified as non-target lesions and indicated as present in the source documents at baseline. The general location will also be documented on the images drawing a regularly-shaped Region of Interest. Measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation.

Those patients, who have no evidence of disease at the baseline, will be documented as no evidence of disease until progression of disease is documented per RECIST 1.1.

- **Complete Response:** Disappearance of all non-target lesions and normalization of tumor marker level, if applicable. All lymph nodes must be non-pathological in size (< 10 mm short axis).
- **Non-Complete Response/Non-Progressive Disease:** Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the upper limits of normal.
- **Progressive Disease:** Appearance of 1 or more new lesions or the unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed at a later time.
    - TUMOR RESPONSE ASSESSMENT BY IRRC – PHASE 2
- Tumor Response will be assessed by the Immune-related response criteria (irRC) as published by Wolchok et al. (68). Response is defined as irCR, irPR or irSD over a period of at least 4 weeks. Disease Control by irRC is defined as irSD or irPR or irCR.

PROGRESSION-FREE SURVIVAL (PFS)

Progression-free survival will be determined for each patient with time origin at the start of the treatment (day 1) until the first occurrence of confirmed progression by irRC or date of death if the patient dies from any causes before progression. Every effort will be made to follow patients for progression after they discontinue the study.

OVERALL SURVIVAL (OS)

Overall survival (OS) will be measured for each patient with time origin at the start of the treatment (day 1) until recorded date of death. Every effort will be made to follow patients for overall survival after they discontinue the study.

Antitumor response based on total measurable tumor burden:

Only index and measurable new lesions are taken into account when evaluating total measurable tumor burden. The longest diameter and longest perpendicular diameter should be recorded in millimeters (mm). At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesion (≥ 10x10 mm; five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (≥ 5 x 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden.

Time-point response assessment:

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening).

irCR

Disappearance of all lesions in two consecutive observations not less than 4 weeks apart.

irPR

≥50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart.

irSD

50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir.

irPD

At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart.

Overall response assessment:

The overall response is derived form time-point response assessment (based on tumor burden) as follows:

Index and new	Non-index non-	New non-measurable	Overall

Measurable lesions (Tumor Burden)	measurable lesions	lesions	Response
100%	Absent	Absent	irCR
100%	Stable	Any	irPR
100%	Unequivocal Progression	Any	irPR
≥50%	Absent/Stable	Any	irPR
>50%	Unequivocal Progression	Any	irPR
<50% TO <25%	Absent/Stable	Any	irSD
<50% TO <25%	Unequivocal Progression	Any	irSD
≥25%	Any	Any	irPD

8.4 Evaluation of Response

Response assessment is for measuring time to disease progression. Time point response assessments will be performed on Cycle 4 Day 1 and Treatment discontinuation (timed to coincide with mid-point and the end of study) with a confirmatory assessment (required for non-randomized trials) within 6 weeks after a PR or CR is deemed. Response assessment may also be based on tumor marker measurements. To determine time point response using imaging studies, refer to **Table 5** and below.

Table 5. Time Point Response Criteria (+/- non-target disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 6. Time Point Response Criteria (non-target disease only)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[1]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

1 Non-CR/non-PD is preferred over SD for non-target disease since SD is used as endpoint for assessment of efficacy in trials so to assign this category when no lesions can be measured is not advised.

The best overall response is the best response recorded from the start of study treatment until end of treatment (taking into account any requirement for confirmation). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria and will be determined by combining the patient's status of target lesions, non-target lesions, and new lesions.

- **Residual Disease:** Patients with objective response but who continue to have residual measurable disease.
- **Symptomatic Deterioration:** Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not related to study treatment or other medical conditions should be reported as progressive disease due to "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment due to symptomatic deterioration. Symptomatic deterioration that may lead to discontinuation of treatment include, but is not limited to, symptoms such as:
  - Weight loss > 10% of body weight.
  - Worsening of disease-related symptoms (e.g., worsening dyspnea, increasing pain/increasing requirement for narcotic analgesics).

- Decline in performance status of > 1 level on ECOG scale.

8.5 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

- **Clinical Lesions:** Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- **Chest x-ray:** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- **Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **PET CT:** At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an Investigator if it is not routinely or serially performed.

- **Ultrasound:** Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for

independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

- **Endoscopy, Laparoscopy:** The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.
- **Tumor Markers:** Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- **Cytology, Histology:** These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

- **FDG-PET:** While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG PET imaging can be identified according to the following algorithm:
    - Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
    - No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
    - FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

9 SAFETY EVALUATION

9.1 Adverse Events

9.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

9.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

9.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

9.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

9.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

9.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. AEs not covered by specific criteria should be reported with common medical terminology and graded according to definitions provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs administered to the patient.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the patient's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably

explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

9.1.3 Reporting Adverse Events

Table 7. Guidelines for Routine Adverse Event Reporting for Phase I Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Table 8. Guidelines for Routine Adverse Event Reporting for Phase IIb Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

All new routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, stabilized, death, or a new treatment is started, whichever comes first, will be reported.

9.2 Serious Adverse Events

9.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a patient in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.2.2 Reporting Serious Adverse Events

All new SAEs occurring from the start of treatment until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. SAE's occurring after this time that the investigator determines to be possibly, probably or definitely related to the study intervention, should be reported.

The Roswell Park SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information. The site Investigator or designated research personnel will report all SAEs, whether related or unrelated, to the investigational agent(s) to the IRB in accordance with their local institutional guidelines.

INCB024360 Cohorts:

Investigators MUST report (within 1 business day), upon becoming aware, to Incyte, Sentrx, and Celldex ANY SAEs, whether or not they are considered related to the investigational agent(s)/intervention.

Incyte Pharmacovigilance

Route 141 and Henry Clay Road

Wilmington, DE 19880

Phone: 302-498-6727

All SAE reports are to be sent via e-mail to: PhVpHVOpsIST@incyte.com

Sentrx

Overlook at Great Notch
150 Clove Road
Little Falls, NJ 07424
SAE Facsimile Number: Sentrx: 866-726-9234
Sentrx Helpdesk Phone (Helpdesk support available 8am – 8 pm EST):
866-278-6759 (toll free in US)
973-812-7575 Ext: 250

Celldex Therapeutics, Inc.
SAE Reporting
Fax No: 781-644-6434
Email: SAE@celldex.com

Exploratory Cohort with sirolimus (Roswell Park site ONLY):

Similarly as above, investigators MUST report (within 1 business day), upon becoming aware, to Sentrx, and Celldex ANY SAEs, whether or not they are considered related to the investigational agent(s)/intervention.

SAE's identified as an Unanticipated Problem by the Investigator must be reported. Please refer to **Section 9.3** for details on reporting Unanticipated Problems.

9.2.3 Follow-Up for Serious Adverse Events

All related SAEs will be followed to their resolution, until the study participant is lost to follow-up, the start of new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

9.3 Unanticipated Problems

9.3.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data.
  b) The characteristics of the patient population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 9.2**.

9.3.2 Reporting Unanticipated Problems

The Reportable New Information (RNI) Form will be submitted to CRS Quality Assurance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Quality Assurance will submit the RNI to the IRB.

When becoming aware of new information about the Unanticipated Problem, submit the updated information to CRS Quality Assurance with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems, whether

related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

9.3.3 Notifying the Study Drug Provider

INCB024360 cohorts:

Expedited reporting by investigator to Celldex, Incyte, and Sentrx. Contact information can be found in section 9.2.2.

The investigator must inform Celldex, Incyte, and Sentrx in writing using an SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied by facsimile within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. An SAE is considered to be any adverse event that is life-threatening or that results in any of the following outcomes: death; inpatient hospitalization or prolongation of existing hospitalization; persistent or significant disability or incapacity; or a congenital anomaly or birth defect. Completed SAE reports are to be submitted to:

Celldex Therapeutics, Inc.
SAE Reporting
Fax No: 781-644-6434
Email: SAE@celldex.com

Exploratory Cohort with Sirolimus:
Similarly as above, expedited reporting by investigator to Celldex and Sentryx.

9.4 FDA Reporting

When Roswell Park is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening.

Or meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A Medwatch for any event that meets the above criteria. Forms will be submitted to the CRS Quality Assurance Office via email to CRSQAGroup@RoswellPark.org.

10 DATA AND SAFETY MONITORING

Phase I studies will be reviewed at the scheduled Roswell Park Early Phase Clinical Trials (EPCT) meetings and the minutes are forwarded to the IRB for review.

The Roswell Park Data Safety Monitoring Committee will assess the progress of the Phase IIb portion of the study, the safety data, and critical efficacy endpoints. The DSMC will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

11 STATISTICAL METHODOLOGY

11.1 Primary Objective

For the Phase I component of the study 2 cohorts will be examined as described in the body of the project description. The following decision rules will be followed: (i) If 0/3 dose limiting toxicities (DLTs) are observed proceed to enroll 3 more patients (ii) If 1/3 DLTs are observed enroll 3 more patients at the same treatment regimen. If 0/6 or 1/6 DLTs are observed, declare the regimen safe and as the maximum tolerated dose combination (MTD) of INCB024360 in combination with the vaccine. If at least 2/3 or 2/6 DLTs are observed in Cohort 1, stop and de-escalate INCB024360 to 100 mg BID in Cohort -1. If at least 2/3 or 2/6 DLTs are observed in Cohort -1, stop and de-escalate INCB024360 to 50 mg BID in Cohort -2. It is anticipated that the probability of a DLT is rare in this setting. A Monte Carlo study using 10,000 replications was conducted. If the Prob(DLT) = 0.2 for Cohort 1 and Prob(DLT) = 0.1 for Cohort -1 or Cohort -2 then the expected sample size for the Phase I component of this study is 5.1 patients and the probability of declaring Cohort 1 as the MTD is 0.71 and the probability of declaring Cohort -1 or Cohort -2 as the MTD is 0.22. If the Prob(DLT) = 0.1 for Cohort 1 and Prob(DLT) = 0.05 for Cohort -1 or Cohort -2 then the expected sample size for the Phase I component of this study is 4.0 patients and the probability of declaring Cohort 1 as the MTD is 0.91 and the probability of declaring Cohort -1 or Cohort -2as the MTD is 0.08. If the Prob(DLT) = 0.5 for Cohort 1 and Prob(DLT) = 0.1 for Cohort -1 or Cohort -2 then the expected sample size for the Phase I component of this study is 6.1 patients and the probability of declaring Cohort 1 as the MTD is 0.17 and the probability of declaring Cohort -1 or Cohort -2 as the MTD is 0.47.

The Phase IIb component of this trial will consist of a randomized parallel design of n = 25 + 25 = 50 patients with treatment arms (i) DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC and (ii) INCB024360 in combination with the DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC . Stratification will be on clinical site (Roswell Park, U. of Pittsburgh). The primary endpoint will be progression free survival. Baseline is defined as the time following the treatment free interval, which can span 0 - 2 months, as described in Iasonos et al.[1] The primary analysis will be carried forth using a Cox proportional hazards model with factors corresponding to treatment and a continuous covariate adjustment for the length of the treatment free interval and a stratification factor for clinical site.Permuted blocked random assignment with block sizes of 4 and an allocation ratio of 1:1 for DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC alone versus INCB024360 in combination with the DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC stratified by clinical site..

11.1.1 Sample Size Justification

We assume a median PFS of 8 months for the INCB024360 alone arm. For the purpose of our simulation study we utilized the simplifying assumption that the covariate adjustment for time between treatment and clinical site are not significant. We also assume a constant hazard rate and a 9 month accrual period with up to 5 years of follow-up. The detectable effect size for the INCB024360 in combination with the DEC205mAB-NY-ESO-1 fusion protein (CDX-1401) arm

different from the INCB024360 alone arm is a PFS of 16 months (or a 2 to 1 risk ratio) with a power estimated from simulation of 0.70. From a practical consideration n = 25 patients per arm is anticipated to be the maximum for which we can accrue during the timeframe of this study.

An additional exploratory cohort (N=6) receiving the same DEC205mAb-NY-ESO-01 fusion protein (CDX-1401) with adjuvant poly-ICLC but in combination with sirolimus in place of INCB024360 will be added to supplement the secondary objectives, which will aid in planning the next phase of the investigation.

11.2 Secondary Objectives

The secondary analysis of continuous immunological response endpoints [antibody titres; NY-ESO-1 specific $CD8^+$ and $CD4^+$ frequency and function (IFN-γ ELISPOT and tumor recognition by CTL)] will be analyzed in a straightforward analysis-of-covariance (ANCOVA) fashion modeling post-treatment levels as a function of pre-treatment levels with factors corresponding to INCB024360 (yes/no) and DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC (yes/no).

Similarly, for the exploratory cohort at Roswell site only, the secondary analysis of continuous immunological response endpoints [antibody titres; NY-ESO-1 specific $CD8^+$ and $CD4^+$ frequency and function (IFN-γ ELISPOT and tumor recognition by CTL)] will be analyzed in a straightforward analysis-of-covariance (ANCOVA) fashion modeling post-treatment levels as a function of pre-treatment levels with factors corresponding to sirolimus (yes/no) and DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC (yes/no).

11.3 Toxicity

All patients enrolled in this study will be eligible for the analysis of toxicity.

The toxicity rate will be estimated using a one-sided, 95%, exact binomial confidence interval (Clopper-Pearson). The lower one sided limit will be used.

11.4 Immune Response

Patients that complete 2 or more cycles of treatment will be eligible for the analysis of immune response.

Due to the early stage of this study and the feasibility considerations, the sample size for this study will be restricted. The analysis of continuous immunological response endpoints (e.g., antibody titers; NY-ESO-1 specific CD8+ and CD4+ frequency and function; frequency of memory T cell populations; TCR avidity) will be analyzed via an analysis-of-covariance (ANCOVA) model with post-treatment levels modeled as a function pre-treatment levels and main effects to the parallel design.

11.5 Interim Analysis and Criteria for Early Termination of the Study

All patients enrolled in this study will eligible for the analysis of safety.

Drug safety will be monitored and evaluated weekly throughout the study including a 30 day safety follow-up period by obtaining, reviewing and analyzing data on AEs, changes in laboratory values, vital signs, and physical examination findings. If the committee views the toxicity as unacceptable, the trial will be stopped early.

In the phase 2 portion of the study, the Roswell Park Data Safety Monitoring Committee will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMC will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

12 CORRELATIVE DATA ANALYSIS

12.1 Pharmacokinetic or Pharmacokinetic/Pharmacodynamic Analysis

A population pharmacokinetic model will be developed utilizing the pharmacokinetic timepoints collected, and then used to estimate individual AUCs or CL of INCB024360 in combination with DEC205mAb-NY-ESO-1 fusion protein (CDX-1401) with adjuvant poly-ICLC vaccine. The effect of patient factors on INCB024360 pharmacokinetics will be evaluated by the model, along with other patient factors that may explain the inter-patient variability in pharmacokinetics. INCB024360 AUC, as well as the observed Cmax, will then be tested for association changes in various immunological biomarkers and the relationship between tryptophan and kynurenine. If an observable trend exists among changes in any of these biomarkers and pharmacodynamic endpoints, a pharmacokinetic/pharmacodynamic model will be developed to evaluate the exposure-response relationship between the time course of INCB024360 exposure (e.g., AUC, Cmax) in relation to changes to these immunological biomarkers. Demographic and clinical data (i.e., ethnicity, current age, age at diagnosis, ECOG status) will be utilized to assess interpatient variability in the model.

13 ETHICAL AND REGULATORY STANDARDS

13.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each patient (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the Investigator to ensure that the patient is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the patient log and patient records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The Investigator is also responsible for obtaining patient authorization to access medical records and other applicable study specific

information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the patient is treated, in accordance with the Declaration of Helsinki, Good Clinical Practice, and according to the guidelines in this protocol, including attached appendices.

13.2 Informed Consent

The Investigator is responsible for obtaining written consent from each patient or the patient's legally authorized representative in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the patient according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The patient should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the information sheet and of the signed consent form to the patient and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the patient file. At any stage, the patient may withdraw from the study and such a decision will not affect any further treatment options.

14 STUDY RESPONSIBILITIES

14.1 Data Collection

Data entry into the database is to be completed in a timely fashion (approximately within 28 days) after the patient’s clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Form, which is handled in an expedited fashion.

Data management activities will be performed using EXPeRT. EXPeRT is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the EXPeRT Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs. EXPeRT is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.

- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the CRC and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

14.2 Monitoring

It is understood that Roswell Park appointed personnel may contact the Investigators, and that they will be allowed direct access to source data/documents for trial related monitoring, audits, IRB Committee review, and regulatory inspection. Direct access is defined as permission to examine, analyze, verify, and reproduce any records and reports that are important to evaluation of a clinical trial. All reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of patients' identities and sponsor's proprietary information will be exercised. (Section 5.15.1 and 1.21, respectively: Guideline for Good Clinical Practice, ICH Harmonized Tripartite Guideline [CPMP/ICH/135/95, adopted July 1996])

It is the appointed personnel's responsibility to inspect the eCRFs at regular intervals throughout the trial to verify adherence to the protocol, the completeness, accuracy and consistency of the data, and adherence to Good Clinical Practice guidelines. The appointed personnel should have access to patient charts, laboratory reports and other patient records needed to verify the entries on the case report forms.

Where local rules do not allow direct access to the source data, the appointed personnel will verify entries in the eCRF by asking direct questions of a person or persons with authorized access to the source data. The Investigator agrees to cooperate with the appointed personnel to ensure that any problems detected during the course of these monitoring visits are resolved.

14.3 Maintenance of Study Documents

Essential documents should be retained per Roswell Park's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with Roswell Park. It is the responsibility of Roswell Park to inform the Investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another Investigator, or to Roswell Park upon written agreement between the Investigator and Roswell Park.

15 ADMINISTRATIVE RULES

15.1 Revisions to the Protocol

Roswell Park may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the CRS/SRC/IRB for written approval before implementation.

15.2 Termination of the Study

It is agreed that, for reasonable cause, the Investigators or Roswell Park may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, Roswell Park may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of patients enrolled in the study.

15.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry will be done using PIDs only. Information will not be released without written authorization of the participant.

16 APPENDICES

Appendix A. ECOG Performance Status Scores

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

Appendix B Sirolimus Drug Interaction List

1. Ketoconazole
2. Posaconazole
3. Voriconazole
4. Itraconazole
5. Erythromycin
6. Clarithromycin
7. Telithromycin
8. Rifampicin
9. Rifabutin
10. Cyclosporine
11. Bromocriptine
12. Diltiazem
13. Metoclopramide
14. Nicardipine
15. Troleandomycin
16. Verapamil
17. Carbamazepine
17. Phenobarbital
18. Phenytoin

Appendix C. Diary for Medication (Poly-ICLC Subcutaneous Injection)

Protocol #: _____ Patient Name: _____

Drug Name: _____ Medical Record #: _____

Cycle (circle one): 1 2 3 4 5

Study Medication (Poly-ICLC Subcutaneous Injection) Calendar for Cohort -2, Cohort -1, Cohort 1, Cohort 2b

Please complete this calendar on a daily basis. Fill in the date for each day in the 1st row and write the time that you give your injection each day in the 2nd row. You should give your injection about 24 hours (±6 hour) from your injection that was given in the clinic.

On days you do not give yourself an injection; please write "NA" in date and time boxes.

Start Date: _____

Day(s) After Vaccine	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date	*No injection will be given on this day.*		*No injection will be given on these days.*				
Time							

Day(s) After Vaccine	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date	*No injection will be given on these days.*						
Time							

Day(s) After Vaccine	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Date	*No injection will be given on these days.*						
Time							

Day(s) After Vaccine	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date	*No injection will be given on these days.*						
Time							

If you missed an injection please comment: _____

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next clinic appointment.

Coordinator's Use Only

Patient Signature: _____ **Date:** _____

CRC Signature: _____ **Date:** _____

ROSWELL PARK COMPREHENSIVE CANCER CENTER
Clinical Research Services

Appendix D. Diary for Medication (INCB024360)

Protocol #: _____ Patient Name: _____

Drug Name: _____ Medical Record #: _____

Cycle (circle one): 1 2 3 4 5 6 7

Study Medication (INCB024360) Calendar for Cohort -2, Cohort -1, Cohort 1, Cohort 2b

Please complete this calendar on a daily basis. Fill in the date for each day in the 1st row, write the drug dose that you take each day in the 2nd row, and write the total number of pills you take each day in the 3rd row. You should take your pills in the morning and in the evening, about 12 hours apart and at least 2 hours after a meal and not have any food for 1 hour after you take your pills.

On days you do not take any study drug; please write "0" in drug dose box. If your dose changes record the new dose level.

Start Date: _____

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Day(s) After Vaccine	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
Date														
Time														
Dose														
Number of pills taken														

	Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14	
Day(s) After Vaccine	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
Date														
Time														
Dose														
Number of pills taken														

	Day 15		Day 16		Day 17		Day 18		Day 19		Day 20		Day 21	
Day(s) After Vaccine	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
Date														
Time														
Dose														
Number of pills taken														

	Day 22		Day 23		Day 24		Day 25		Day 26		Day 27		Day 28	
Day(s) After Vaccine	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
Date														
Time														
Dose														
Number of pills taken														

If you missed a dose please comment:_____

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

____% $Compliance = \left(\frac{\quad}{\quad}\right) \times 100$ **Patient Signature:** _____

Date: _____

CRC Signature: _____ **Date:** _____

ROSWELL PARK COMPREHENSIVE CANCER CENTER
Clinical Research Services

Appendix E Diary for Medication (Sirolimus)

Study No.: I 248613 **Patient Name:** _____

Drug Name: Sirolimus **Medical Record No.:** _____

Cycle (circle one): 1 2 3 4

Study Medication (Sirolimus) Calendar for Exploratory Cohort

Please complete this calendar on a daily basis immediately after you take your tablets. Fill in the date for each day in the 1st row, write the time of day that you take each day in the 2nd row (circle AM or PM), and write the total number of tablets you take that day in the 3rd row.

On days you do not take any study drug; please write "0" in the "Number of tablets taken" box.

Day(s):After Vaccine	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
Time	AM PM	AM PM	AM PM	AM PM	AM PM	AM PM	AM PM
Number of tablets taken							

Day(s):After Vaccine	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date							
Time	AM PM	AM PM	AM PM	AM PM	AM PM	AM PM	AM PM
Number of tablets taken							

Day(s):After Vaccine	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Date	**No Drug Should Be Taken On These Days.**						
Time							
Number of tablets taken							

Day(s) :After Vaccine	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date	**No Drug Should Be Taken On These Days.**						
Time							
Number of tablets taken							

If you missed a dose please comment: _____

Please remember to bring this calendar and your tablet bottle (including any unused tablets) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$\_\_\_\%\ Compliance = \left(\frac{\quad - \quad}{\quad\quad\quad\quad}\right) \times 100$$

Patient Signature: _____ Date: _____

CRC signature: _____ Date: _____

Investigator signature: _____ Date: _____

Appendix F. Immunohistochemistry to Determine NY-ESO-1 Tumor Expression

1. Adhere 5 μm paraffin sections to charged slides (Fisherbrand Superfrost/Plus Slides). Five sections are required.
2. Heat slides in 60°C oven for 2 hours.
3. Deparaffinize slides in xylene and bring to water through graded alcohols.
4. Antigen Retrieval: Steam heat slides by immersing slides in a 90°C, 1:10 solution of DAKO's Target Retrieval Solution High pH for 15 minutes.
5. Cool at room temperature for 30 minutes.
6. Perform IHC; Block tissue with 5% BSA/PBS solution for 30 minutes at room temperature.
7. Apply primary antibody (in this case ES121 [2.5 μg/mL]) overnight, 4°C.
8. Rinse with PBS.
9. Apply secondary; (Vector, Burlingame, CA) Horse anti mouse, 1:200; 30 minutes at room temperature.
10. Rinse with PBS.
11. Block endogenous peroxidase with a 1% H2O2 solution for 20 minutes at room temperature.
12. Rinse with PBS.
13. Apply avidin-biotin-complex system (Vector) for 30 minutes at room temperature.
14. Rinse with PBS.
15. Develop with diaminobenzidine tetrahydrochloride (DAB, Biogenex, San Ramon, CA).
16. Counterstain with hematoxylin solution.
17. Dehydrate and cover slip.

Appendix G. Nucleic Acid Determination of NY-ESO-1 and LAGE-1 Tumor Expression

Determination of NY-ESO-1 and LAGE-1 tumor expression by reverse transcription (RT) of total RNA and polymerase chain reaction (PCR) amplification of cDNA

RT-PCR Reagents:

25 mM $MgCl_2$ Solution (Perkin Elmer-Cetus)

10 x PCR Buffer II, pH 8.3 (Perkin Elmer-Cetus)

dNTPs:

dGTP, 10 mM]
dATP, 10 mM] Mix 1:1:1:1 before use (Perkin Elmer-Cetus)
dTTP, 10 mM]
dCTP, 10 mM]

RNasin RNAse Inhibitor (40 µg/mL) (Promega)

M-MLV Reverse Transcriptase (200 µg/µL) (Gibco BRL)

Random Hexamer (400 ng/µL) (Promega)

Primers:

ESO1A and ESO1B primers (each at a concentration of 200 ng/µL)

5' primer ESO1-A1: CAGGGCTGAATGGATGCTGCAGA

3' primer ESO1-B1: GCGCCTCTGCCCTGAGGGAGG

Anticipated size of PCR amplified product = 329 bp.

For LAGE-1 typing, the primer sequences are

5' primer LAGE-1A: CTGCGCAGGATGGAAGGTGCCCC

3' primer LAGE-1B: GCGCCTCTGCCCTGAGGGAGC

Anticipated size of PCR amplified product = 332 bp.

Amplitaq Gold DNA Polymerase (5 µg/µL) (Perkin Elmer-Cetus)

Nuclease-Free Water (Promega)

Mineral Oil (Perkin Elmer-Cetus)

Other Supplies:

0.5 mL sterile microcentrifuge tubes

Barrier or Aerosol Resistant Pipet Tips

Reverse Transcriptase (RT) Protocol

1. Set up each RT sample in a sterile 0.5 mL microcentrifuge tube. Use only Aerosol Resistant Pipet Tips, changing tips after each use to prevent cross-contamination. A master mix consisting of all the above reagents minus the RNA can be made and then aliquotted appropriately.

Component	Initial Concentration	Volume	Final Concentration
$MgCl_2$	25 mM	4 µL	5 mM
PCR Buffer II	10X	2 µL	1X
DNTP	Mix (1:1:1:1)	8 µL	1 mM each of: dGTP, dATP, dTTP, CTP
RNAse Inhibitor	40 ng/µL	1 µL	2 ng (per 20 µL)
M-MLV Reverse Transcriptase		1 µL	10 µg (per 20 µL)
Random Hexamer		1 µL	400 ng (per 20 µL)
Total RNA in Nuclease Free Water		3 µL	2 µg (per 20 µL)
Final		20 µL	

2. Overlay the above RT sample mix with mineral oil to reduce evaporation or refluxing. Incubate the RT sample mix at room temperature for 10 minutes for the random hexamer to anneal and begin reverse transcription of the RNA.

3. Place a drop of mineral oil into each well of the thermal cycler that is to be used. This will ensure even heating and cooling of each sample during the RT cycle. Incubate all tubes in a thermal cycler according to the following parameters:

Step Cycle			
Segment 1	42°C	60 minutes	Stable annealing of the random hexamer for reverse transcription
Segment 2	99°C	5 minutes	Inactivation of M-MLV Reverse Transcriptase
Segment 3	5°C	5 minutes	
Cycle Count 1			
Link to			
Soak Cycle	4°C		

Polymerase Chain Reaction (PCR) Protocol

1. Set up each PCR sample in a sterile 0.5 mL microcentrifuge tube. Use only Aerosol Resistant Tips, changing tips after each use to prevent cross-contamination. A negative control (without cDNA) should be run with each PCR panel to ensure the purity of the reagents. A master mix of all reagents, minus the cDNA, can be prepared when running several PCR samples at once.

Component	Initial Concentration	Volume	Final Concentration or Amount
$MgCl_2$	25 mM	2 µL	2 mM
PCR Buffer II	10X	2.5 µL	1X
DNTP	Mix (1:1:1:1)	2 µL	0.2 mM
ESO-1A1[a]	200 ng/µL	0.25 µL	50 ng (per 25 µL)
ESO-1B1[a]	200 ng/µL	0.25 µL	50 ng (per 25 µL)
Amplitaq Gold DNA Polymerase	5 µg/µL	0.125 µL	0.625 µg (per 25 µL)
Nuclease Free Water		12.875 µL	—
CDNA		5 µL	0.5 µg
Final		25 µL	

[a] For LAGE-1 methods, replace ESO-1A1 and ESO-1B1 with LAGE-1A and LAGE-1B, respectively.

2. Overlay the above PCR sample mix with sterile mineral oil to reduce evaporation or refluxing.

3 Place a drop of mineral oil into each well of the thermal cycler. This will ensure even heating and cooling of each sample during the PCR cycle. Incubate tubes in a thermal cycler according to the following parameters:

Time Delay Cycle	95°C	10 min	Initial heatup for Activation of Amplitaq Gold DNA polymerase
Cycle count 1 Link to *Step cycle*			
Segment 1	94°C	1 minute	Denaturation of cDNA
Segment 2	60°C	1 minute	Primer annealing
Segment 3	72°C	1 minute	Primer extension
Cycle Count 35 Link to *Time Delay Cycle*	72°C	6 minute	Final Primer extension
Link to *Soak Cycle*	4°C		

Gel Electrophoresis

Use 15 µL of the 25 µL PCR product, analyze by 1.2% to 1.5% agarose gel (ethidium bromide in the gel).

GENERAL COMMENTS

1. RNA Preparation

Guanidium thiocyanate/CsCl gradient method is used to prepare RNA. Other RNA protocols, such as Trizol, should give comparable results. The RNA preparations should be quantitated by measuring absorbance at 260 nm.

Absorbance at 260 x dilution factor x 40 mcg/mL = concentration (μg/mL)

Confirm the RNA and cDNA quality by control trans-intron primers for any housekeeping gene, preferentially with anticipated PCR product > 350 bp – 400 bp. Usually a faint background smear on the gel is visible even when the RNA being analyzed is NY-ESO-1 negative In the case of an absolutely clean gel on electrophoresis it may signify a poor PCR, probably due to poor RNA template or poor RT.

2. RT-PCR

Perform the RT reaction with 2 mcg RNA per 20 mL reaction Mixture. Use 5 μL of this RT product for 25 μL PCR reaction, corresponding to 0.5 mcg total RNA. Use 15 μL of the PCR product for electrophoresis.

For PCR reaction, AmpliTaq Gold gave more consistent results than other Taq polymerase and should be used.

Since some RNA preparations generate a nonspecific band slightly smaller than the NY-ESO-1 product comparison with a positive control is necessary. Testicular RNA or RNA from a known NY-ESO-1 positive line is an appropriate positive control. Using the above protocol 1 μL cDNA prepared from testicular RNA should give a clear positive signal.

3. Gel Electrophoresis

Short run for 1 to 3 hours provides better results than overnight gel at low voltage, particularly for weaker positives.

Results are considered positive if a 332 bp band is noted for NY-ESO-1 and 338 bp is noted LAGE-1.

As a positive internal control all PCR reactions are done using testicular RNA.

Appendix H. Shipping Information for HLA Testing

HLA specimen collection kit containing 3 ten ml EDTA purple top tubes provided by Roswell Park.

Collect should occur at baseline if HLA typing has not already performed.

Specimens should be shipped via Prime Time Courier, ambient on the same day of collection, with enclosed Immco specimen requisition. This will be handled by Roswell Park Lab medicine department and shipped to:

Immco Diagnostics
640 Ellicott Street
Buffalo, NY 14203

Appendix I. Assessment of T cell Responses

$CD4^+$ and $CD8^+$ cells will be enriched from PBMCs by magnetic cell sorting using miniMACS (Miltenyi Biotec Inc., Bergisch Gladbach, Germany) and stimulated with irradiated autologous APCs in the presence of NY-ESO-1 peptide pool (each 2 μM; NeoMPS Inc., San Diego, CA), rhIL-2 (10 IU/mL), and rhIL-7 (10 ng/mL). At Day 8, cultures will be tested for intracellular IFN-γ secretion after stimulation, during 4 hours, and in the absence or presence of the peptide pool or of individual peptides. Response will be considered significant if the frequency of T cells detected in at least one post vaccine sample exceeded by 3-fold that found in the baseline sample.

For ex vivo assessments, cryopreserved total PBMCs will be thawed, rested overnight, and stimulated for 7 hours in the absence or presence of the NY-ESO-1 peptide pool. Brefeldin A will be added 2 hours after the beginning of the incubation. At the end of the incubation, cells will be stained with antibodies directed against surface markers (CD3, CD4, CD8, CD45RA, and CCR7), fixed, permeabilized, and stained with cytokine-specific antibodies.

Appendix J. Instructions for Network Sites

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:

Roswell Park
CRS Network Office
ASB K 104
Buffalo, New York 14263

Telephone:
Monday - Friday; 7:00 AM to 4:00 PM EST
716-845-8084

After hours, weekends, and holidays request the Roswell Park Investigator
716-845-2300

Fax: 716-845-8743

1. INFORMED CONSENT

- Informed consent must be obtained by the **site Investigator** from any subjects wishing to participate, **prior to any procedures or change in treatment**.
- An informed consent template is provided by Roswell Park and can be amended to reflect institutional requirements.
- All consent changes **must** be reviewed by Roswell Park Network Office prior to submission to the site IRB.
- The informed consent must be IRB approved.
- Always check that the most up to date version of the IRB approved consent is being used.
- Within 5 business days, notify the Roswell Park Network Office of all participant withdrawals or consent to limited study participation and appropriately document the discontinuation and the reason (s) why.

2. SUBJECT REGISTRATION

The subject completes the **Gender, Race, and Ethnicity Form** and this is placed in the study binder.

Roswell Park does not grant exceptions to eligibility criteria.

Phase 2 Protocol Registration Instructions

The **Subject Enrollment Log** must be faxed or emailed to the Roswell Park Network Office within 24 hours of the date the subject is consented. Once the Investigator has determined that eligibility has been met, complete the **Subject Registration Form and fax or email** it to the Roswell Park Network Monitor at 716-845-8743.

3. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this must be reported to the Roswell Park Network, site IRB and any other regulatory authority involved in the trial.
- All study deviation will be recorded on the **Study Deviation Log**.
- Subjects inadvertently enrolled with significant deviation(s) from the study-specified criteria will be removed from the study.

4. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The Roswell Park Network Monitor must be able to read what has been deleted.
    - Do **NOT** use white-out, magic marker, scratch-outs.
    - Do **NOT** erase entries.
- Use only black ink for documentation on the accountability form and any other study forms.

5. DRUG ACCOUNTABILITY

Drug accountability must be strictly maintained.

- Responsibility rests solely with the Investigator but can be delegated as appropriate (e.g., to pharmacy personnel).
- A drug accountability record form (DARF) will record quantities of study drug received, dispensed to subjects and wasted, lot number, date dispensed, subject ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.
- Study drug supply will only be used in accordance with the IRB approved study.
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.

- An inventory count must be performed with each transaction. Any discrepancies shall be documented and explained.
- Drug accountability forms must be stored with study related documents.
- Each medication provided for this study and each dosage form and strength must have its own DARF.
- Dispensing the wrong study supply is considered a **medication error**.
    - **NEVER** replace investigational agents with commercial product.
    - Do **NOT** "transfer", "borrow" or "replace" supplies between studies.

6. SERIOUS ADVERSE EVENT REPORTING

The site Investigator or designated research personnel will report all SAEs, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**. The site will notify the Roswell Park Network Monitor within 1 business day of being made aware of the SAE. A preliminary written report must follow within 24 hours (1 business day) of the first notification using the following forms:

- Roswell Park SAE report form
- MedWatch 3500A

Investigators MUST report (within 1 business day), upon becoming aware, to Incyte, Sentrx, or Celldex ANY SAEs, whether or not they are considered related to the investigational agent(s)/intervention.

Sentrx
Overlook at Great Notch
150 Clove Road
Little Falls, NJ 07424
SAE Facsimile Number: Sentrx: 866-726-9234
Sentrx Helpdesk Phone (Helpdesk support available 8am – 8 pm EST):
866-278-6759 (toll free in US)
973-812-7575 Ext: 250

Incyte Pharmacovigilance
Route 141 and Henry Clay Road
Wilmington, DE 19880
Phone: 302-498-6727
All SAE reports are to be sent via e-mail to: PhVpHVOpsIST@incyte.com

Celldex Therapeutics, Inc.
SAE Reporting
Fax No: 781-644-6434

E-mail: SAE@celldex.com

A complete follow-up report must be sent to the Roswell Park Network Monitor within 10 working days.

7. UNANTICIPATED PROBLEM REPORTING

An unanticipated problem (UP) is any incident, experience, or outcome that meets **all** of the criteria in **Section** 9.3

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention, the participating physician or delegated research staff from each site will notify **their local IRB in accordance with their local institutional guidelines**. The site must also notify the Roswell Park Network Monitor within 24 hours of being made aware of the Unanticipated Problem by completing the **Roswell Park Reportable New Information Form** and faxing or emailing it to the Roswell Park Network Monitor.

8. LAB INFORMATION

Baseline Tumor Tissue Requirements

- Evaluation of all other biomarkers will occur in the Pathology Network Shared Resource at Roswell Park.
    - 8 unstained sections (5µm thick) on plus glass

Send samples, along with the sponsor-provided shipping log, using study-specific subject ID number and tissue accession number to Roswell Park Pathology Network Shared Resource (Attn: Protocol Lab Team). The

Shipping label should read as follows:
Roswell Park
Correlative Sciences Pathology Office, S-636
Attn: Protocol Lab Team – I 248613 Samples
Elm & Carlton Streets
Buffalo, NY 14263
(716) 845-8917
Email: CRSLabPathTeam@RoswellPark.org and the network monitor

NY-ESO-1 Tissue Testing for Eligibility at baseline

- Pathologist at the institution where the patient had surgery should choose a block of representative tumor with sufficient tumor nuclei and little necrosis.
- Either send the block or cut:
- 5 unstained slides at 4-5 um on charged glass slides, air dried and
- 8 tissue curls- cut with molecular precautions at 10 um and placed in a tube or on non-charged slides)

- If there is not enough material for both the 4-5 um and the 10 um curls, please send just the slides and we test by IHC only. The slides and curls can be sent with a cold pack
- Label the samples with patient ID, patient initials and study number
- Complete the OmniSeq Order Requisition form and provide a copy of the pathology report (de-identified with patient initial and study ID #)
- Specimen Packing Sheet needs to be filled out and sent with samples.
- Tissue must be shipped from Magee Hospital – U Pitt

Shipping of NY-ESO-1 Tissue Testing for Eligibility at baseline

Ship samples Monday – Thursday (not on a holiday) using standard overnight delivery
OmniSeq, LLC
700 Ellicott Street
Buffalo NY 14203

Receiving phone is 716-898-8654

Notify via email: specimenreceiving@omniseq.com and jennifer.ventola@roswellpark.org with Site name, FedEx tracking # and Study #, Pathology Report and OmniSeq Req.

HLA Typing

- HLA specimen 3 (10mL) Lavender Top blood at Screening.
- Ship whole blood ambient same day of collection **priority overnight through FedEx**
  IMMCO Diagnostics
  Immunogenetics Laboratory
  640 Ellicott Street
  Buffalo, NY 14203
- Roswell Park Research requisition should be included with sample for each patient. This form is called **Immco_HLA_testing**
- These will be collected and shipped only Monday- Thursday (no holidays):

Notify via email: Ckillion@immco.com and Network Monitor (jennifer.ventola@roswellpark.org)
Include the FedEx #, patient ID, and Study Number (see "Immco_HLA_Testing" form for shipping address)

Immunological Response:

70 mL of blood for immunological analysis will be collected using 6 green top and 1 red top.
Collected on visits:
Cycle 1 Day 1
Cycle 2 Day 1
Cycle 3 Day 1
Cycle 4 Day 1
Cycle 5 Day 1
Treatment Discontinuation
3 month, 6 month, 12 month after last vaccine or dose of INCB

Overnight ship the samples at room temp to:
Immune Analysis Facility
Cancer Cell Center, Room 416
Roswell Park
Elm and Carlton Streets
Buffalo, NY 14263
Attn: Junko Matsuzaki, PhD

When shipping samples make sure to include the **shipping log.

NOTIFY via email Junko (junko.matsuzaki@roswellpark.org) and jennifer.ventola@roswellpark.org the FedEx #, patient ID, **Time point,** and Study number

Correlative studies

Whole blood samples for pharmacokinetic analysis of **INCB024360 levels** will be collected via venipuncture using (2) 2 mL purple-top EDTA collection tube per time point. (Make sure they are draw one right after each other)

Cycle 1 Day 15: predose and 2 hours postdose of INCB024360
Cycle 2 Day 1: trough, prior to vaccination

-Plasma will be separated by centrifugation at 4°C from whole blood within 30 minutes following the extraction and aliquoted into (2) cryovials per time-point.
-The tube will be labeled with the participant's number, patient's initials, clinical study number, protocol time point, dose, and protocol day.

The samples will be immediately frozen at -70ºC or below until analyzed.
Samples will be processed in Roswell Park's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility.

Shipping of Samples will occur at least once a year or 50% of the site accrual is met.
***Ship Monday – Thursday for next day delivery (Not on a holiday)

When shipping samples make sure to include the **shipping log.

Address:
Roswell Park
Bioanalytics, Metabolomics & Pharmacokinetics Core Facility,
Center for Genetics and Pharmacology, Room L1-140,
Study # I 248613,
Elm & Carlton Streets,
Buffalo, New York 14262,

Notify via email: PKPDCore@RoswellPark.org and jennifer.ventola@roswellpark.org : with Your site name, FedEx # and study #

Correlative studies … cont'd

Whole blood samples for pharmacokinetic analysis of **tryptophan and kynurenine levels** will be collected via venipuncture using (2) 2 mL purple-top EDTA collection tube per time point. (Make sure they are draw one right after each other)

Cycle 1 Day 15: predose and 2 hours postdose of INCB024360
Cycle 2 Day 1: trough, prior to vaccination

Plasma will be separated by centrifugation at 4°C from whole blood within 30 minutes following the extraction and aliquotted into (2) cryovials per time-point.
-The tube will be labeled with the participant's number, patient's initials, clinical study number, protocol time point, dose, and protocol day.

The samples will be immediately frozen at -70ºC or below until analyzed.
Samples will be processed in Roswell Park's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility.

Shipping of Samples will occur at least once a year or 50% of the site accrual is met.
***Ship Monday – Thursday for next day delivery (Not on a holiday)

When shipping samples make sure to include the **shipping log.

Address:
Roswell Park
Bioanalytics, Metabolomics & Pharmacokinetics Core Facility,
Center for Genetics and Pharmacology, Room L1-140,
Study #: I 248613,
Elm & Carlton Streets,
Buffalo, New York 14262,

Notify via email: PKPDCore@RoswellPark.org and jennifer.ventola@roswellpark.org : with Your site name, FedEx # and study #

17 REFERENCES

1. Iasonos, A., et al., *Identifying clinical improvement in consolidation single-arm phase 2 trials in patients with ovarian cancer in second or greater clinical remission.* Int J Gynecol Cancer. **22**(1): p. 63-9.
2. Ries, L.A.G., C.L. Kosary, and B.F. Hankey, *Cancer statistics review 1973-1996.* NCI, 1999.
3. Greenlee, R.T., et al., *Cancer statistics, 2001.* CA Cancer J Clin, 2001. **51**(1): p. 15-36.
4. Engel, J., et al., *Moderate progress for ovarian cancer in the last 20 years: prolongation of survival, but no improvement in the cure rate.* Eur J Cancer, 2002. **38**(18): p. 2435-45.
5. Armstrong, D.K., et al., *Intraperitoneal cisplatin and paclitaxel in ovarian cancer.* N Engl J Med, 2006. **354**(1): p. 34-43.
6. Chen, Y.T., et al., *A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening.* Proc Natl Acad Sci U S A, 1997. **94**(5): p. 1914-8.
7. Odunsi, K., et al., *NY-ESO-1 and LAGE-1 Cancer-Testis Antigens Are Potential Targets for Immunotherapy in Epithelial Ovarian Cancer.* Cancer Res, 2003. **63**(18): p. 6076-83.
8. Chen, Y.T., et al., *Genomic cloning and localization of CTAG, a gene encoding an autoimmunogenic cancer-testis antigen NY-ESO-1, to human chromosome Xq28.* Cytogenet Cell Genet, 1997. **79**(3-4): p. 237-40.
9. Lethe, B., et al., *LAGE-1, a new gene with tumor specificity.* Int J Cancer, 1998. **76**(6): p. 903-8.
10. Bonifaz, L.C., et al., *In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination.* J Exp Med, 2004. **199**(6): p. 815-24.
11. Bozzacco, L., et al., *DEC-205 receptor on dendritic cells mediates presentation of HIV gag protein to CD8+ T cells in a spectrum of human MHC I haplotypes.* Proc Natl Acad Sci U S A, 2007. **104**(4): p. 1289-94.
12. Figdor, C.G., Y. van Kooyk, and G.J. Adema, *C-type lectin receptors on dendritic cells and Langerhans cells.* Nat Rev Immunol, 2002. **2**(2): p. 77-84.
13. Tsuji, T., et al., *Antibody-Targeted NY-ESO-1 to Mannose Receptor or DEC-205 In Vitro Elicits Dual Human CD8+ and CD4+ T Cell Responses with Broad Antigen Specificity.* J Immunol, 2010.
14. Munn, D.H., *Indoleamine 2,3-dioxygenase, tumor-induced tolerance and counter-regulation.* Curr Opin Immunol, 2006. **18**(2): p. 220-5.
15. Fallarino, F., et al., *The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells.* J Immunol, 2006. **176**(11): p. 6752-61.
16. Curiel, T.J., et al., *Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival.* Nat Med, 2004.
17. Hou, D.Y., et al., *Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses.* Cancer Res, 2007. **67**(2): p. 792-801.
18. Lob, S., et al., *Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells.* Blood, 2007.

19. Lob, S., et al., *IDO1 and IDO2 are expressed in human tumors: levo- but not dextro-1-methyl tryptophan inhibits tryptophan catabolism.* Cancer Immunol Immunother, 2009. **58**(1): p. 153-7.
20. Qian, F., et al., *Efficacy of levo-1-methyl tryptophan and dextro-1-methyl tryptophan in reversing indoleamine-2,3-dioxygenase-mediated arrest of T-cell proliferation in human epithelial ovarian cancer.* Cancer Res, 2009. **69**(13): p. 5498-504.
21. Yue, E.W., et al., *Discovery of potent competitive inhibitors of indoleamine 2,3-dioxygenase with in vivo pharmacodynamic activity and efficacy in a mouse melanoma model.* J Med Chem, 2009. **52**(23): p. 7364-7.
22. Liu, X., et al., *Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity.* Blood, 2010. **115**(17): p. 3520-30.
23. Liu, X., et al., *Selective inhibition of indoleamine 2,3-dioxygenase (IDO1) effectively regulates mediators of anti-tumor immunity.* Blood.
24. Koblish, H.K., et al., *Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors.* Mol Cancer Ther. **9**(2): p. 489-98.
25. Abraham, R.T. and J.J. Gibbons, *The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy.* Clin Cancer Res, 2007. **13**(11): p. 3109-14.
26. Cho, D., et al., *The role of mammalian target of rapamycin inhibitors in the treatment of advanced renal cancer.* Clin Cancer Res, 2007. **13**(2 Pt 2): p. 758s-763s.
27. Hudes, G., et al., *Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma.* N Engl J Med, 2007. **356**(22): p. 2271-81.
28. Bjornsti, M.A. and P.J. Houghton, *The TOR pathway: a target for cancer therapy.* Nat Rev Cancer, 2004. **4**(5): p. 335-48.
29. Del Bufalo, D., et al., *Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus.* Cancer Res, 2006. **66**(11): p. 5549-54.
30. Hidalgo, M., et al., *A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer.* Clin Cancer Res, 2006. **12**(19): p. 5755-63.
31. Raymond, E., et al., *Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer.* J Clin Oncol, 2004. **22**(12): p. 2336-47.
32. Araki, K., et al., *mTOR regulates memory CD8 T-cell differentiation.* Nature, 2009. **460**(7251): p. 108-12.
33. Rao, R.R., et al., *The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin.* Immunity, 2010. **32**(1): p. 67-78.
34. Sato, E., et al., *Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer.* Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18538-43.
35. Nishikawa, H., et al., *Influence of CD4+CD25+ regulatory T cells on low/high-avidity CD4+ T cells following peptide vaccination.* J Immunol, 2006. **176**(10): p. 6340-6.
36. Zhang, L., et al., *Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer.* N Engl J Med, 2003. **348**(3): p. 203-13.

37. Ino, K., *Indoleamine 2,3-dioxygenase and immune tolerance in ovarian cancer.* Curr Opin Obstet Gynecol, 2011. **23**(1): p. 13-8.
38. Ino, K., et al., *Indoleamine 2,3-dioxygenase is a novel prognostic indicator for endometrial cancer.* Br J Cancer, 2006. **95**(11): p. 1555-61.
39. Inaba, T., et al., *Role of the immunosuppressive enzyme indoleamine 2,3-dioxygenase in the progression of ovarian carcinoma.* Gynecol Oncol, 2009. **115**(2): p. 185-92.
40. Okamoto, A., et al., *Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells.* Clin Cancer Res, 2005. **11**(16): p. 6030-9.
41. Takao, M., et al., *Increased synthesis of indoleamine-2,3-dioxygenase protein is positively associated with impaired survival in patients with serous-type, but not with other types of, ovarian cancer.* Oncol Rep, 2007. **17**(6): p. 1333-9.
42. Odunsi, K., et al., *Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer.* Proc Natl Acad Sci U S A, 2007. **104**(31): p. 12837-42.
43. Matsuzaki, J., et al., *Recognition of naturally processed and ovarian cancer reactive CD8(+) T cell epitopes within a promiscuous HLA class II T-helper region of NY-ESO-1.* Cancer Immunol Immunother, 2008. **57**(8): p. 1185-95.
44. Matsuzaki, J., et al., *Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer.* Proc Natl Acad Sci U S A, 2010. **107**(17): p. 7875-80.