Abbreviated Title: AdHER2/neu DC Vaccine
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Title: A Phase I Study of an Adenoviral Transduced Autologous Dendritic Cell Vaccine Expressing Human HER2/neu ECTM in Adults with Tumors with 1-3+ HER2/neu Expression

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Investigational Agent:

Agent: Autologous dendritic cells transduced with an adenoviral vector: Ad5f35 human HER2/*neu* ECTM (AdHER2)

Drug Name:	Ad5f35HER2ECTM transduced autologous dendritic cell vaccine
IND Number:	15212
Sponsor:	Center for Cancer Research
Manufacturer:	Department of Transfusion Medicine, NIH Clinical Center

Commercial Agents: None

Identifying words: Human epidermal growth factor receptor 2 (HER2) expression, dendritic cell vaccine breast cancer, stomach cancer, metastatic tumors

PRÉCIS

Background:

- Human epidermal growth factor receptor 2 (HER2, also known as c-*erb*B2 or *neu*) is a proto-oncogene that encodes a 185-kd transmembrane (TM) tyrosine kinase receptor that participates in receptor-receptor interactions that regulate cell growth, differentiation and proliferation. Its over-expression contributes to neoplastic transformation.
- HER2 is over-expressed in up to 25-30% of node-positive or node-negative primary breast cancers and is associated with clinically aggressive breast cancer, a high recurrence rate and reduced survival.
- Trastuzumab (Herceptin®) is a recombinant humanized mouse monoclonal antibody (MAb) that binds to the extracellular (EC) domain of the HER2 receptor. Its clinical efficacy is limited to patients with 3+ HER2 tumor expression documented by immunohistochemistry (IHC) or a HER2 amplified tumor by Vysis fluorescent *in situ* hybridization (FISH). IHC is a subjective measurement of HER2/*neu* protein while FISH is an objective measurement of amplification of the HER2 oncogene.
- Although the use of trastuzumab has been associated with improved clinical outcomes, a significant number of patients are unresponsive to therapy and most eventually experience clinical progression. At present, no vaccine is available that induces patients to make their own anti-HER2 antibodies.
- We propose to investigate the use of an adenoviral vector (Ad5f35) expressing *human* HER2ECTM (Ad5f35HER2ECTM- AdHER2) to transduce autologous dendritic cells for therapeutic vaccination in patients with HER2 expressing solid tumors.

Objectives:

- To determine the safety and toxicity of autologous AdHER2 dendritic cell vaccination.
- Specifically, to determine if the fraction of patients with cancer therapeutics-related cardiac dysfunction (CTRCD), defined as a decrease in LVEF ≥10 percentage points, to a value LVEF to ≥53% (normal reference value for two-dimensional (2-D) echocardiography), is sufficiently low to warrant further development in subsequent trials.
- To determine the immunogenicity of autologous AdHER2 dendritic cell vaccination as measured by a 3-fold increase in anti-HER2/neu antibody concentration or a 4-fold increase in antibody dilution titers over baseline.

Eligibility:

- Adults ≥ 18 of age
- Patients with 1+ to 3+ HER2/neu expression by IHC or a Vysis FISH result equivocal or positive.
- Part I: Naïve to trastuzumab (Herceptin™), pertuzumab (Perjeta™) and lapatinib (Tykerb™), ado-trastuzumab emtansine (Kadcyla™) or other HER2-directed therapies.
- Part II: Recurrent or progressive metastatic disease after standard of care HER2- targeted therapies with known clinical benefit; i.e. trastuzumab (Herceptin™), pertuzumab

(Perjeta™), lapatinib (Tykerb™), ado-trastuzumab emtansine (TDM1) (Kadcyla™) or other HER2-directed therapies.

- Eligible malignancies:
    - Parts I and II: Malignant soft tissue and bone tumors and recurrent or progressive, metastatic solid tumors who have progressed on standard therapies (Note: Patients with metastatic cancer must have measurable disease per RECIST 1.1); or,
    - Part I only: Bladder cancer in the adjuvant setting (adjuvant bladder cancer patients):
        - Tumor stage T3a, T3b, T4a, T4b and any node positive disease regardless of tumor stage.
        - Status-post primary cystectomy with curative intent.
        - May or may not have received neoadjuvant cisplatin-based combination chemotherapy per NCCN guidelines.
        - May or may not have received adjuvant radiotherapy or chemotherapy based on pathologic risk per NCCN guidelines.
        - Greater than or equal to 6 weeks status post primary surgery with curative intent.
- Adequate hematologic, metabolic, hepatic, renal, and cardiac function

Study Design:

Open label, non-randomized, two-part, phase I study of 48 weeks duration for evaluation of primary endpoints with extended follow-up out to 30 months to monitor LVEF cardiac function.

Part I involves vaccine dose escalation in a *population with* ***no prior exposure*** *to trastuzumab or other HER2-targeted therapies* to determine if there is a significant, adverse safety signal regarding cardiac toxicity, in addition to preliminary assessment of the vaccine's immunogenicity and clinical activity in patients with metastatic solid tumors or high-risk bladder cancer in the adjuvant setting. Response will be evaluated by a Modified Immune-Related Response Criteria (modified irRC) based on Response Evaluation Criteria in Solid Tumors (RECIST 1.1).

Part II is identical to part I in the schedule of treatment and response evaluation, but is conducted in a population with **prior exposure** to trastuzumab and other HER2-targeted therapies.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the safety and toxicity of autologous AdHER2/neu dendritic cell vaccination. Specifically, to:
    - Determine if the fraction of patients with cancer therapeutics-related cardiac dysfunction (CTRCD), (if it occurs) is sufficiently low to warrant further development in subsequent trials. CTRCD is defined as a decrease in LVEF ≥ 10 percentage points, to a value ≥ 53% (normal reference value for two-dimensional (2-D) echocardiography)[1].
- To determine the immunogenicity of autologous AdHER2 dendritic cell vaccination as measured by a 3-fold increase in anti-HER2/neu antibody concentration (measured as mcg/mL) or a 4-fold increase in antibody dilution titers over baseline.

1.1.2 Secondary Objectives

- To determine the preliminary activity of autologous AdHER2 dendritic cell vaccination as measured by the fraction of subjects who have stable disease, a partial response or better by modified immune-related response criteria.
- To determine the impact of autologous AdHER2 dendritic cell vaccination on tumor growth rate and regression rate constants.
- To characterize vaccine-induced antibody profiles using HER2 peptide microarrays, examining reactivity to HER2 extracellular (EC), transmembrane (TM) and intracellular (IC) domains.
- To characterize and measure function-associated mRNAs in whole blood, circulating tumor cells, myeloid derived suppressor cells (MDSCs) and other potential biologic/immunologic correlates of clinical response throughout treatment.

1.2 BACKGROUND AND RATIONALE

1.2.1 HER2 Directed Therapies

Human epidermal growth factor receptor 2 (HER2, also known as c-*erb*B2 or *neu*) is a proto-oncogene that encodes a 185-kd transmembrane (TM) tyrosine kinase receptor that participates in receptor-receptor interactions that regulate cell growth, differentiation and proliferation. Its over-expression- either HER2 oncogene amplification and/or over-expression of the HER2 protein, contributes to neoplastic transformation[2]. HER2 is over-expressed in up to 25-30% of node-positive or node–negative primary breast cancers and is associated with clinically aggressive breast cancer, a high recurrence rate and reduced survival[3]. Development of agents targeting HER2 has resulted in expansion of therapeutic options and improved clinical outcomes for patients with HER2 over-expressing tumors[4]. Current FDA-approved treatment options include trastuzumab and lapatinib. A third agent, pertuzumab is under priority review by the FDA with a decision expected by June 8th, 2012 for a proposed indication in combination with trastuzumab and docetaxel chemotherapy in patients with HER2-positive metastatic or locally

recurrent, unresectable breast cancer, who have not received previous treatment or whose disease has relapsed after adjuvant therapy.

1.2.1.1 Trastuzumab (Herceptin®)

Trastuzumab (Herceptin®) is a recombinant humanized mouse monoclonal antibody (MAb) that binds to the extracellular (EC) domain of the HER2 receptor and was approved by the FDA in September 1998. It is approved for the adjuvant treatment of early-stage breast cancer with nodal spread (or without nodal spread if the tumor is high risk) as well as for first and second-line/salvage therapy for metastatic breast cancer[5]. In the adjuvant setting trastuzumab is used in several ways:

- As part of a treatment course including chemotherapy with doxorubicin, cyclophosphamide and either paclitaxel or docetaxel, known as "ACTH".
- With chemotherapy drugs docetaxel and carboplatin in a regimen known as "TCH".
- Alone after treatment with multiple other therapies, including anthracycline-based therapy.

Trastuzumab has two approved uses in metastatic breast cancer:

- As first line treatment in combination with paclitaxel.
- As a single agent in patients who have received one or more courses of chemotherapy for metastatic disease.

Therapy typically involves an initial trastuzumab dose of 4mg/kg IV followed by 2mg/kg IV administered weekly for 52 weeks, alone (salvage therapy) or in combination with paclitaxel for the first 12 weeks (adjuvant and first-line therapy). However, clinical efficacy is limited to patients with 3+ HER2 tumor expression documented by immunohistochemistry (IHC) or gene amplification by fluorescent *in situ* hybridization (FISH). IHC is a subjective measurement of HER2/*neu* protein while FISH is an objective measurement of amplification of the HER2 oncogene[6]. Despite the high level efficacy in combination with chemotherapy, the overall response rate of women diagnosed with HER2-positive metastatic breast cancer and treated with trastuzumab as a single first-line agent is only 26%[7] clearly indicating there is room for improvement in therapeutic options. In addition, a significant number of patients are unresponsive to trastuzumab and most eventually experience clinical progression while on therapy, presumably due to trastuzumab resistance, whose mechanism is poorly understood[8-10].

Trastuzumab is also approved for use in combination with cisplatin and either capecitabine or 5-fluorouracil in treatment naïve patients with HER2-positive metastatic stomach cancer or cancer of the gastroesophageal junction.

Despite the success of trastuzumab, no vaccine is currently available that induces patients to make their own anti-HER2 antibodies.

1.2.1.2 Trastuzumab Cardiac Toxicity

Given the well-documented cardiac toxicity associated with the monoclonal antibody trastuzumab [11-13] we believe it is critical to initially assess the safety of the vaccine as a single agent to minimize confounding attributions of toxicity. Specifically, a retrospective review of records for patients enrolled onto any of seven phase II and III trastuzumab clinical trials revealed patients treated with trastuzumab were found to be at increased risk for cardiac dysfunction[11]. The incidence was greatest in patients receiving concomitant trastuzumab and anthracycline plus cyclophosphamide (27%) but was substantially lower in patients receiving

paclitaxel and trastuzumab (13%) or trastuzumab alone (3% to 7%) although most of these patients had received prior anthracycline therapy. Most trastuzumab-treated patients developing cardiac dysfunction were symptomatic (75%), and most improved with standard treatment for congestive heart failure (79%).

Consequently, we propose to conduct this study in two parts to identify any cardiac safety issues that may be associated with vaccine-induced antibodies. Part I of the study involves vaccine dose escalation in a *population with **no prior exposure** to trastuzumab* or other HER2 directed agents to determine if there is a significant, adverse safety signal regarding cardiac toxicity. Assessments of cardiac function will include serial echocardiography to determine left ventricular ejection fraction (LVEF) at baseline and at regular monitoring intervals, in addition to LVEF measurements for at least 2 years following receipt of the last dose of vaccine. Once cardiac safety has been established in this population, Part II repeats the vaccine dose escalation *in a population with **significant prior exposure** to trastuzumab* to determine whether there is an adverse safety signal regarding cardiac toxicity using identical monitoring and follow-up criteria. Please note for patients in parts I and II with taking known CT agents will no longer be on study. This study design and monitoring plan for cardiac toxicity as well as thresholds for discontinuation of vaccine treatment were developed based on FDA recommendations from the Pre-IND meeting review on September 6th, 2011.

1.2.1.3 Lapatinib (Tykerb™)

Lapatinib is an orally administered tyrosine kinase inhibitor of both the HER2/*neu* protein and the epidermal growth factor receptor. It has shown activity in combination with capecitabine in patients with metastatic HER2 positive breast cancer that have progressed following treatment with trastuzumab[14]. Common toxicities specific to lapatinib include diarrhea and rash; cardiac toxicity is rarely seen. Like trastuzumab, resistance to lapatinib has been described and it appears that multiple molecular mechanisms underly resistance to both of these drugs[15].

1.2.1.4 Pertuzumab (Perjeta™)

Pertuzumab is a humanized monoclonal antibody being studied in early and advanced states of HER2-positive breast cancer and advanced HER2-positive gastric cancer. It is a dimerization inhibitor designed to specifically prevent the HER2 receptor from pairing (dimerizing) with other HER receptors (EFGR/HER1, HER3 and HER4), a process that is believed to play a critical role in the growth and formation of several different cancer types. Through prevention of receptor pairing, pertuzumab is believed to block cell signaling, which may inhibit cancer cell growth or lead to the death of the cancer cell. Binding of pertuzumab to HER2 may also signal the body's immune system to destroy cancer cells. The mechanisms of action of pertuzumab and trastuzumab are thought to complement each other, as both bind to the HER2 receptor but on different regions. The goal of combination pertuzumab/trastuzumab therapy and chemotherapy is to determine if the combination provides a more comprehensive blockade of HER signaling pathways.

The pertuzumab application before the FDA is based on results from the pivotal phase III CLEOPATRA (**Cl**inical **E**valuation **O**f **P**ertuzumab **A**nd **TRA**stuzumab) study[16]. CLEOPATRA is an international, phase III, randomized, double-blind, placebo-controlled study. The study evaluated the efficacy and safety profile of the pertuzumab-based regimen compared to trastuzumab and chemotherapy plus placebo in 808 people with previously untreated HER2-positive metastatic breast cancer. The primary endpoint of the study was progression free

survival (PFS) with secondary endpoints of overall survival (OS), PFS by investigator assessment, safety profile, overall response rate (ORR), duration of response, time to symptom progression and correlation of biomarkers with clinical outcomes.

The CLEOPATRA study demonstrated a 6.1-month improvement in median PFS for patients receiving a pertuzumab-based regimen (pertuzumab combined with trastuzumab and docetaxel) compared to those who received trastuzumab and docetaxel alone: median PFS 18.5 vs. 12.4 months. People who received the combination also experienced a 38% reduction in the risk of disease worsening or death (HR=0.62, p-value <0.0001). The pertuzumab regimen was not associated with a higher incidence of cardiac AEs: left ventricular dysfunction was 4.4% in the pertuzumab-containing regimen compared to 8.3% with trastuzumab and docetaxel alone. Final OS analysis from the CLEOPATRA study is expected in 2013.

1.2.1.5 Trastuzumab Emtansine (T-DM1) (Kadcyla™)

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate consisting of trastuzumab linked via a non-reducible thiotether to the cytotoxic, anti-tubulin agent maytansine (DM1)[17]. It is under global development by Roche in collaboration with Genentech and Immunogen for second-line, first-line and third-line treatment of HER2+ metastatic breast cancer in the following phase III trials, respectively:

- The EMILIA study will compare T-DM1 versus lapatinib plus capecitabine in 980 patients (NCT00829166.)
- The MARIANNE study will compare docetaxel/trastuzumab vs. T-DM1 vs. T-DM1 plus pertuzumab (NCT01120184).
- The TH3RESA study is comparing T-DM1 versus best supportive care in the salvage setting (NCT01419197).

The results of a 137 patient, randomized phase II study of T-DM1 in untreated HER2+ metastatic breast cancer patients comparing T-DM1 versus trastuzumab plus docetaxel chemotherapy presented in September 2011 at the European Multidisciplinary Cancer Congress in Stockholm Sweden (Abstract #5001) demonstrated improved median PFS of T-DM1 compared to trastuzumab/docetaxel: median PFS of 14.2 months vs. 9.2 months, respectively; p=0.035. T-DM1 achieved a 64.2% objective response rate vs. 58% for the trastuzumab/docetaxel combination and also had a more favorable side effect profile, with a lower frequency of severe adverse events (SAEs): 46.4% vs. 89.4% respectively. The most frequent SAEs reported with T-DM1 therapy were thrombocytopenia (8.7%) and elevated liver transaminases (8.7%). Data from the EMILIA trial is expected in 2012 and is anticipated to be the basis for seeking FDA marketing approval for second-line treatment of HER2+ cancer in the U.S. and Europe. Ado-trastuzumab emtansine (Kadcyla) was approved by the FDA on February 22, 2013.

1.2.2 Pre-Clinical Animal Studies

We have recently published dramatic results documenting the regression and cure of large established tumors in syngeneic BALB/c mice using a therapeutic adenoviral vector vaccine expressing the extracellular and transmembrane (ECTM) domains of rodent HER2*neu*[18]. This anti-tumor activity is mediated by *polyclonal vaccine-induced antibodies* that inhibit HER2 phosphorylation and unlike trastuzumab, are *Fc receptor independent*.

Although many mouse tumor vaccine studies have prevented growth of tumors, it has generally not been possible to achieve cure of large established tumors > 1 cm in diameter with cancer vaccines. Using an adenoviral vector vaccine expressing the extracellular and transmembrane (ECTM) domains of rodent HER2/*neu*, we have cured TUBO mammary gland carcinomas up to 2 cm in diameter in syngeneic BALB/c mice (**Figure 1**) and multiple large established lung metastases (**Figure 2**). Virtually 100% of mice are cured from both subcutaneous and lung tumors. The vaccine also prevents spontaneous orthotopic breast carcinomas in BALB/c mice transgenic for the same rodent n*eu* oncogene[18,19].

Figure 1

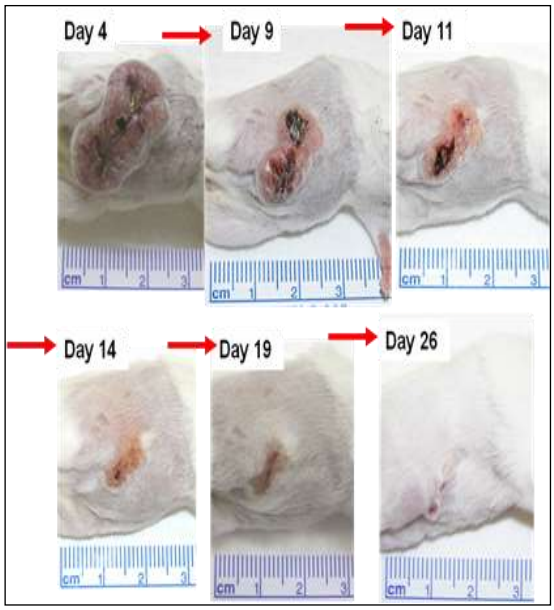
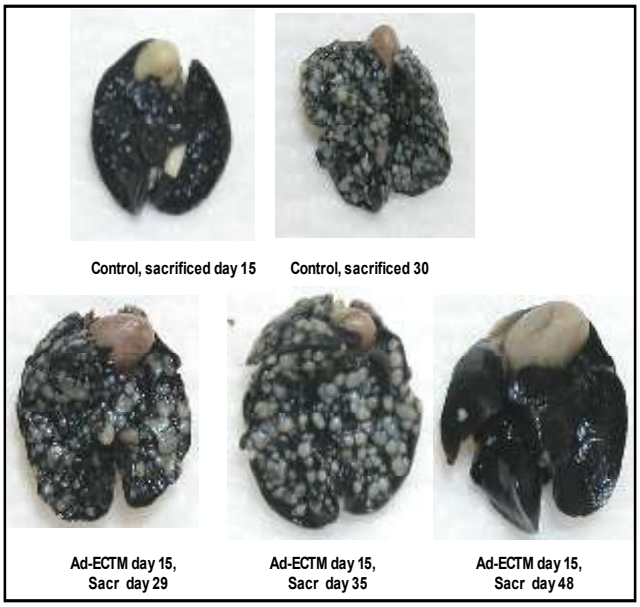


Figure 2



A single vaccination of 10^8 pfu of Ad*neu*ECTM causes regression of large (> 1 cm^2) tumors as demonstrated in **Figure 3** and **Figure 4**.

Figure 3: Ad*neu*ECTM Causes Regression of Large Tumors

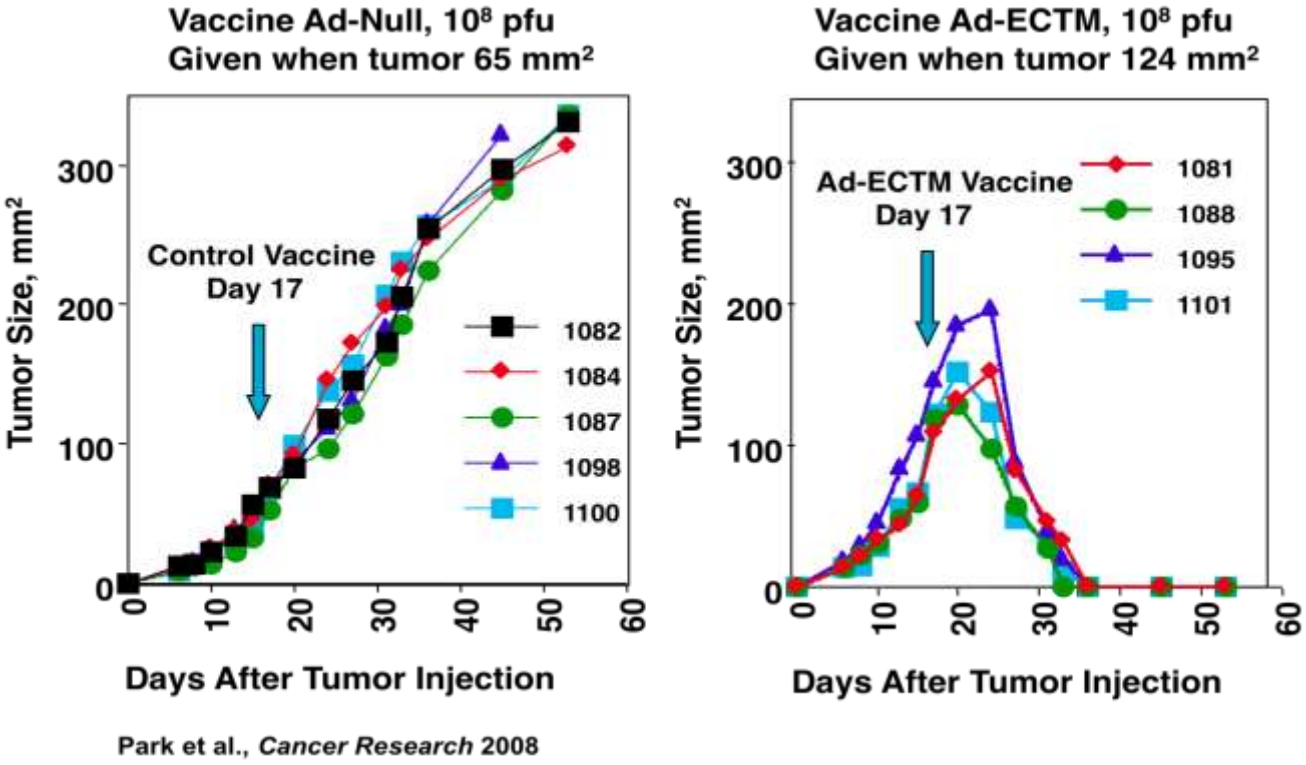


Figure 4: Large Breast Tumors are Cured with a Single Injection of Ad*neu*ECTM

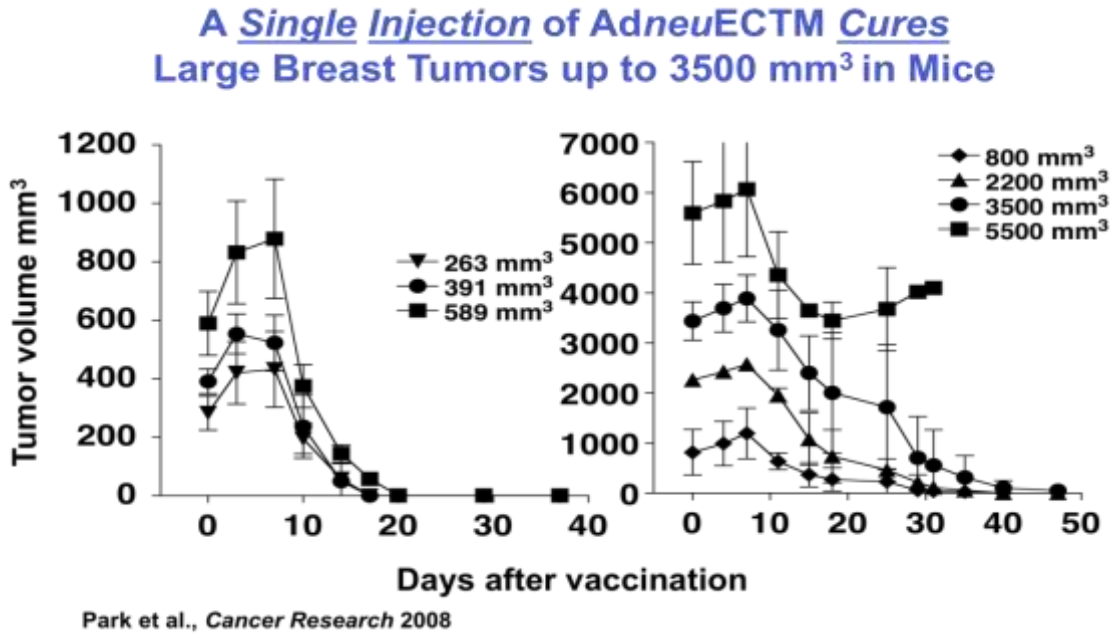
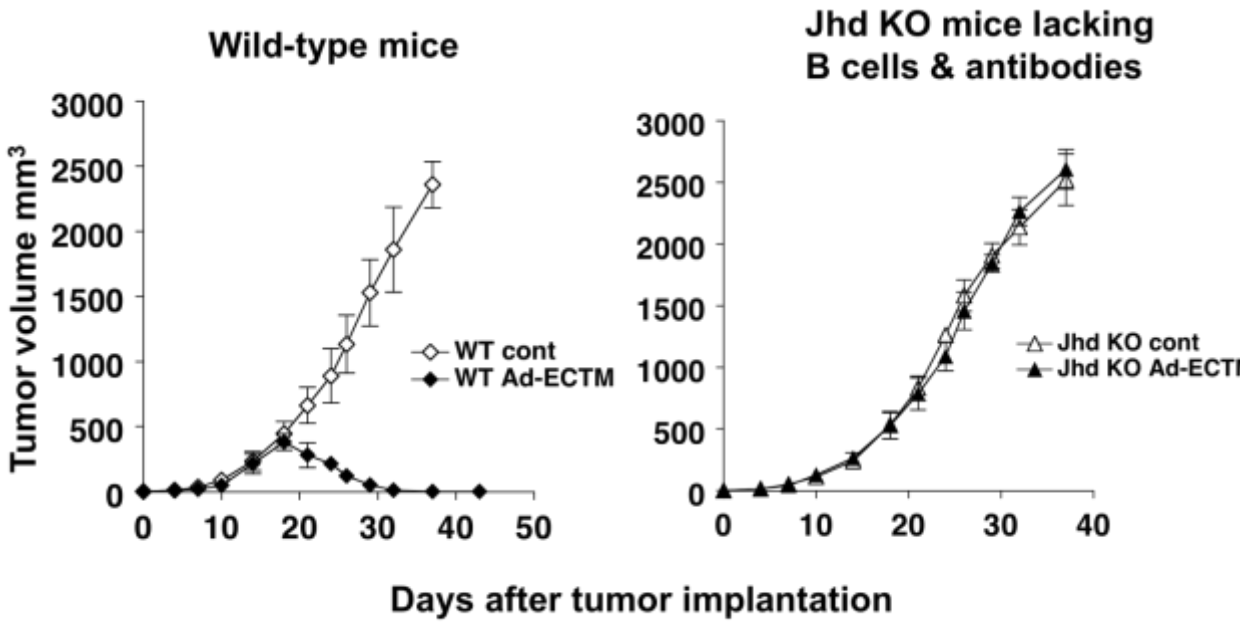


Figure 5: Ad*neu*ECTM Vaccine Therapy is ***Antibody Dependent***



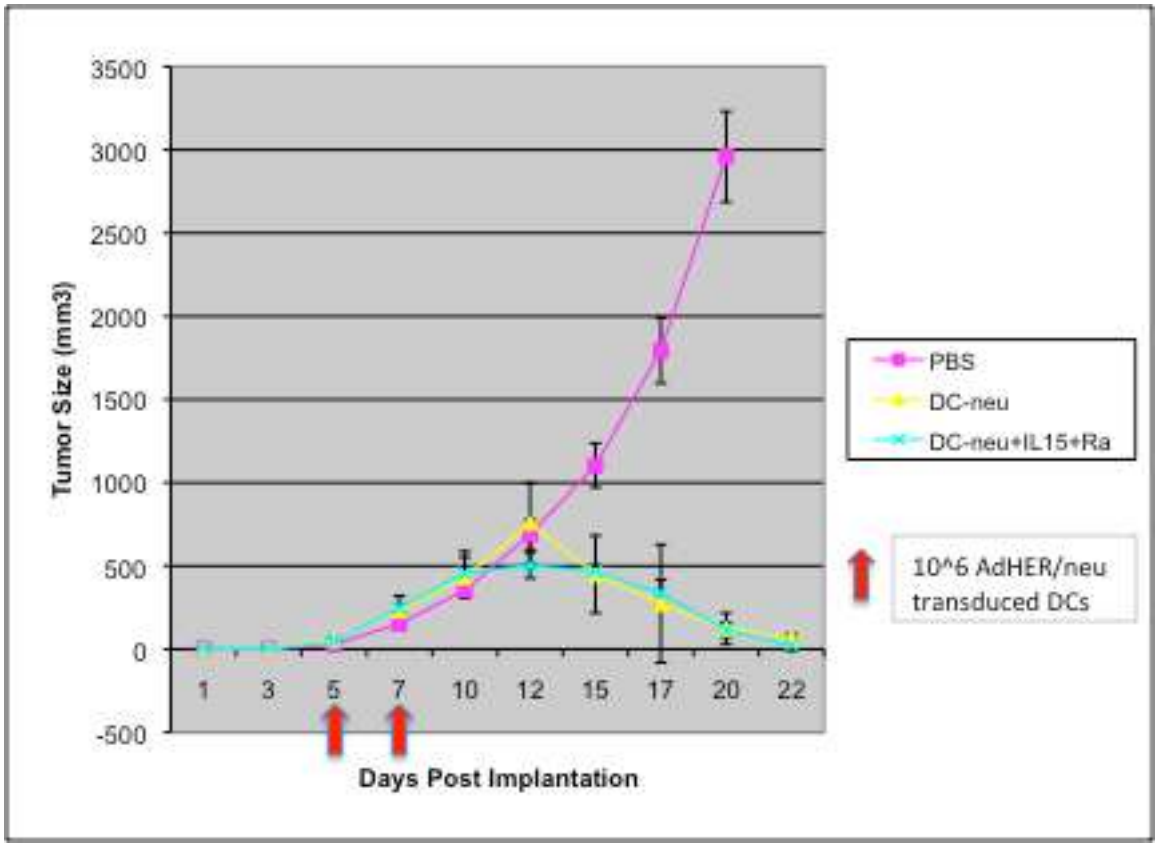
Park et al., *Cancer Research* 2008

As shown above in **Figure 5**, the vaccine's mechanism of action is purely antibody-mediated, with no involvement of CD4 or CD8 T cells in the effector function (only CD4 help at the time of vaccination to induce antibodies). Neither depletion of $CD8^+$ T cells with antibody nor use of beta-2 microglobulin knockout mice that lack $CD8^+$ T cells affects vaccine-induced protection.

1.2.3 Rodent AdneuECTM DC Vaccination

Similar induction of therapeutically effective antibodies could be obtained by immunizing with 1 million syngeneic dendritic cells transduced with the Ad-neuECTM vector as shown below in **Figure 6**. Mice given TUBO cells subcutaneously and then vaccinated on days 5 and 7 when the tumors were 55-250 mm^3 showed complete regression of tumors exactly analogous to the regressions induced by the free AdHER2 vector (Steele, Morris et al, unpublished observations). Tumors continued to grow to a peak around Day 12, when they reached a maximum of 756 mm^3, and then started to regress, reaching baseline levels after Day 22. This therapeutic effect was observed whether Ad*neu*ECTM-transduced DCs were given alone or with IL-15 and was also antibody dependent. Thus, immunization with Ad*neu*ECTM-transduced DCs and with free Ad*neu*ECTM both produced comparable antibody-mediated tumor regression. Also, sera from such Ad*neu*ECTM-transduced DC-immunized mice inhibited growth of TUBO cells *in vitro* and inhibited phosphorylation of the ErbB2-neu oncoprotein.

Figure 6: **Rodent Ad*neu*ECTM DC Vaccination**



1.2.4 Central and Peripheral Tolerance in the Transgenic HER2 Mouse Model

It should be noted that HER2/neu-transgenic mice have central or thymic tolerance to the oncogene, which is expressed from before birth (unlike some models in which an oncogene is not expressed until sexual maturity). They express a smaller, more limited and different T cell repertoire from that in non-transgenic mice, reflecting the deletion of many T cell clones by central self-tolerance[20,21]. Thus, it is not surprising that it has been harder to treat established spontaneous tumors in the *neu*-transgenic mice[19,22]. Further, in the BALB-*neu* transgenic model, the oncogene is expressed in all mammary cells of all mammary glands. As a consequence, relentless malignant transformation of mammary gland cells is induced with subsequent generation of independent primary tumors in all 10 mouse mammary glands, making it extremely difficult to suppress all tumors. In contrast, it is still possible to virtually completely suppress the development of tumors in neu-transgenic mice if vaccination is started at a young age, about 7 weeks, when histopathology already shows development of abnormal mammary gland dysplasia (beginning even before 4 weeks) due to the oncogene. Presumably the vaccine-induced antibodies are eradicating or inhibiting the dysplastic neu-expressing cells. Thus, this is really a therapeutic model, not just prophylactic. This situation contrasts with the human breast cancer situation, in which the HER2/*neu* oncogene is not expressed until malignant transformation in adulthood, so there is no opportunity to develop central tolerance. Indeed, Disis and coworkers[23,24] have demonstrated that many patients with HER2+ breast cancers

express spontaneous antibodies and T cell responses to the HER2 oncoprotein, demonstrating the absence of central tolerance. Further, humans with breast cancer generally do not have such an oncogene expressed in 100% of mammary gland ductal epithelial cells as in the transgenic mice, leading to the relentless and continuous generation of new independent tumors. Therefore, it is expected that the vaccine will be more effective at inducing therapeutically effective antibodies to HER2 in patients with $HER2^+$ breast cancers than it can in centrally tolerant neu-transgenic mice.

We have also shown (unpublished data) that vaccine-induced antibodies inhibit the growth of the N202-1A tumor line, another mouse mammary carcinoma expressing the same oncogene. The vaccine's mechanism of action is purely antibody-mediated, with no involvement of CD4 or CD8 T cells in the effector function (only CD4 help at the time of vaccination to induce antibodies). Importantly, the vaccine-induced antibodies work differently from trastuzumab: they are *Fc receptor independent*, kill tumor cells directly without the aid of other cells, and inhibit HER2 phosphorylation and cause it to be modulated off the cell surface. Thus, this vaccine may work in patients in whom trastuzumab or lapatinib is ineffective. In addition, *vaccine-induced antibodies* are *polyclonal* and may be more resistant to escape mutations than a monoclonal antibody (MAb) like trastuzumab or pertuzumab. Our pre-clinical animal data suggest the potential for even greater therapeutic efficacy than trastuzumab and would eliminate the need for costly, frequent infusions currently associated with this treatment.

1.2.5 Pre-Clinical AdHER2ECTM Vector Expression Testing

Several rounds of pre-clinical experiments have been conducted to determine the optimal conditions for generation of AdHER2 transfected dendritic cells (AdHER2 DC) for autologous therapeutic vaccination. Healthy normal donor cells were used to generate dendritic cells under different conditions to confirm HER2*neu* expression by FACS or ELISA in multiple experiments and functional assays which were performed by Drs. Luciano Castiello and Marianna Sabatino in the Department of Transfusion Medicine (DTM) and by Dr. Masaki Terabe of the Vaccine Branch. The key outcomes from these experiments are documented below:

- Equivalent HER2neu expression of DCs transfected under bag or flask conditions. The DTM currently uses bags to generate DCs from thawed monocytes for autologous DC vaccines as part of their cGMP standard operating procedures and this is the methodology that will be used in our study.
- A viral particle (VP): DC ratio of 3000:1 was chosen as the ratio for transfection for this clinical trial since high level expression was documented at reasonable MOIs based on data highlighted in the tables below.

Table 1 FACS Data HER2neu Expressions Varying VP: DC Ratios Round 2 M. Sabatino

VP:DC Ratio	Population	Expression Marker	# Events	% Parent
1000:1	FITC G1 HER2	HER2	6,947	26.8
2000:1	FITC G1 HER2	HER2	20,571	79.4
3000:1	FITC G1 HER2	HER2	24,657	95.0
5000:1	FITC G1 HER2	HER2	24,073	99.5

- HER2*neu* mean fluorescent intensity and the percent of cells staining positive for HER2*neu* was acceptable at the two highest ratios examined (M. Terabe FACS data):

VP:DC Ratio	Mean Fluorescent Intensity (MFI)	% Cells Positive (DY and N87) for HER2*neu*
3000:1	103	84.5%
5000:1	432	97.8%

- Although MFI and percent positive cells were even greater at 5000:1, there were concerns about potential subsequent issues of cell viability at this highest ratio. In addition, the efficient vialing of vector for planned MOIs of 3000:1 will allow an adequate supply of virus for up to 66% more patients than if the 5000:1 ratio were to be used.

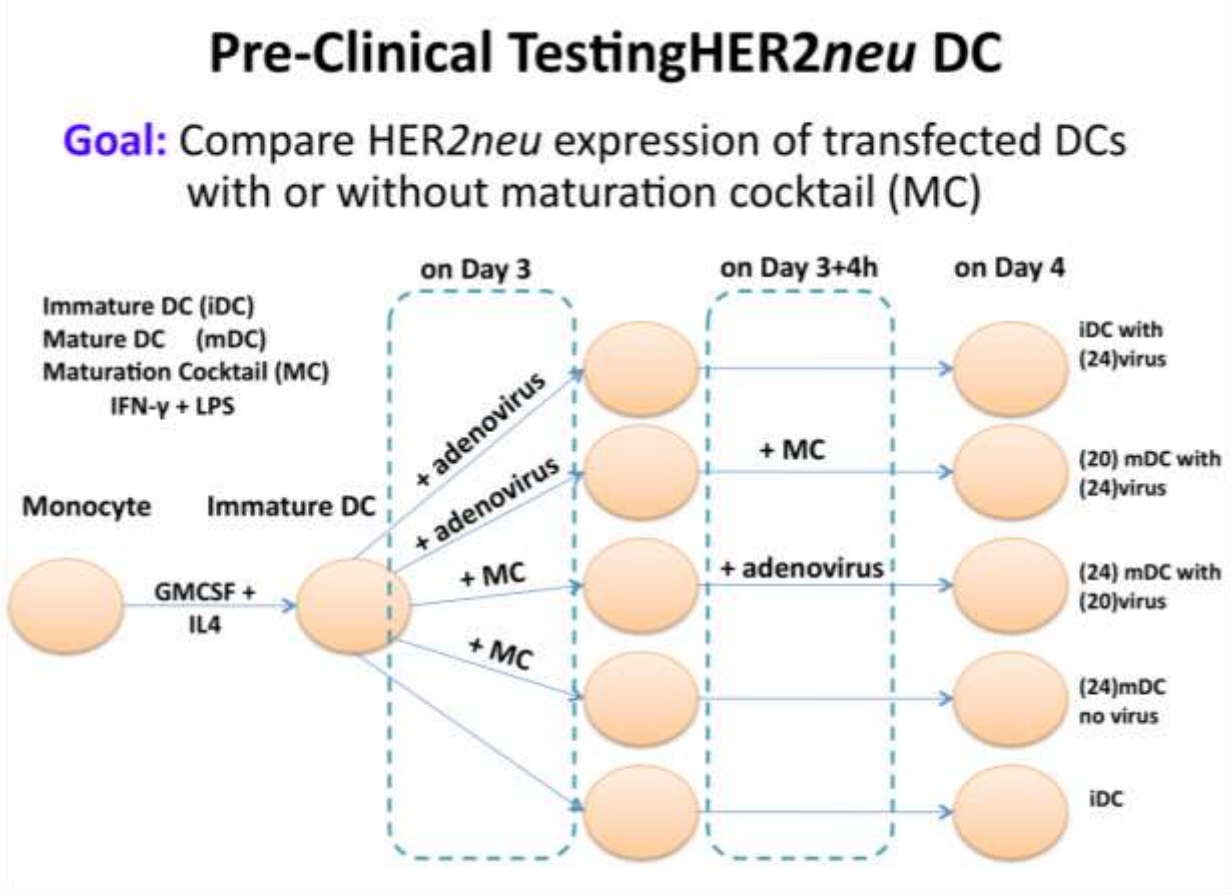
The above data were reviewed by and discussed with the FDA in a Pre-IND meeting conference call held September 6th, 2011 and were determined to be acceptable.

1.2.6 Pre-Clinical HER2 Dendritic Cell Platform Testing

Studies were conducted utilizing cells from three healthy, normal human donors (11FC00039, 11FC00043 and 11FC00055) to determine the best sequence of AdHER2 vector transfection and dendritic cell maturation for optimal viability and HER2*neu* expression in the final DC vaccine product. AdHER2 transfected DCs at a ratio of 3000 VP: DC were generated under the following conditions as summarized below and outlined in **Figure 7:**

- Transfection of immature dendritic cells (iDC) for 24 hours: **iDC with (24h)virus**
- Immature dendritic cells with no viral transfection: **iDC**
- Maturation of DCs for 24 hours with *no viral transfection*: **(24h)mDC no virus**
- Maturation of DCs for 24 hours with transfection at 20 hours: **(24h)mDC with(20h)virus**
- Transfection of DCs for 4 hours followed by maturation of DCs: **(20h)mDC with(24h)virus**

Figure 7: DC Transfection and Maturation Experiment



DC viability and product yield (expressed as % of starting number of monocytes) under the varying transfection and DC maturation conditions are outlined in **Figure 8** and **Figure 9.**

Figure 8

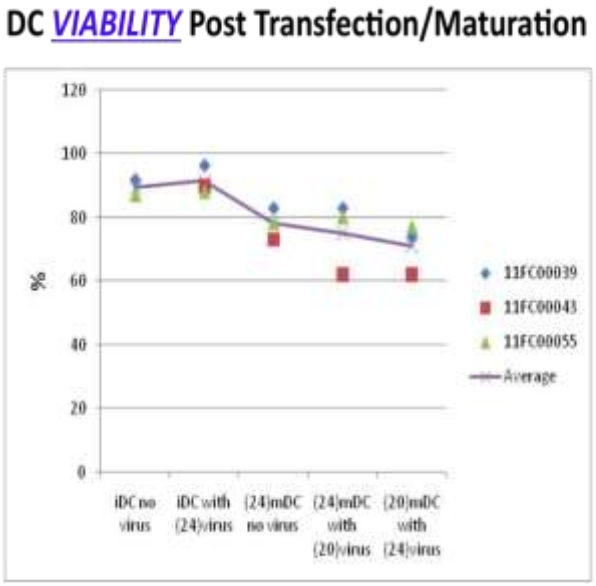
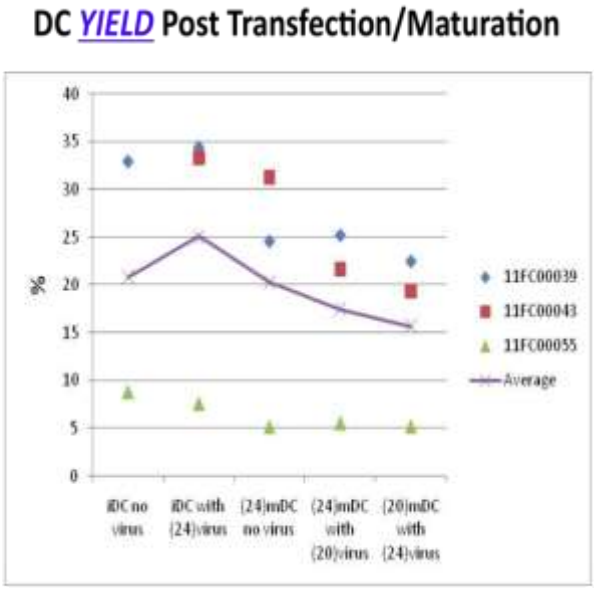


Figure 9

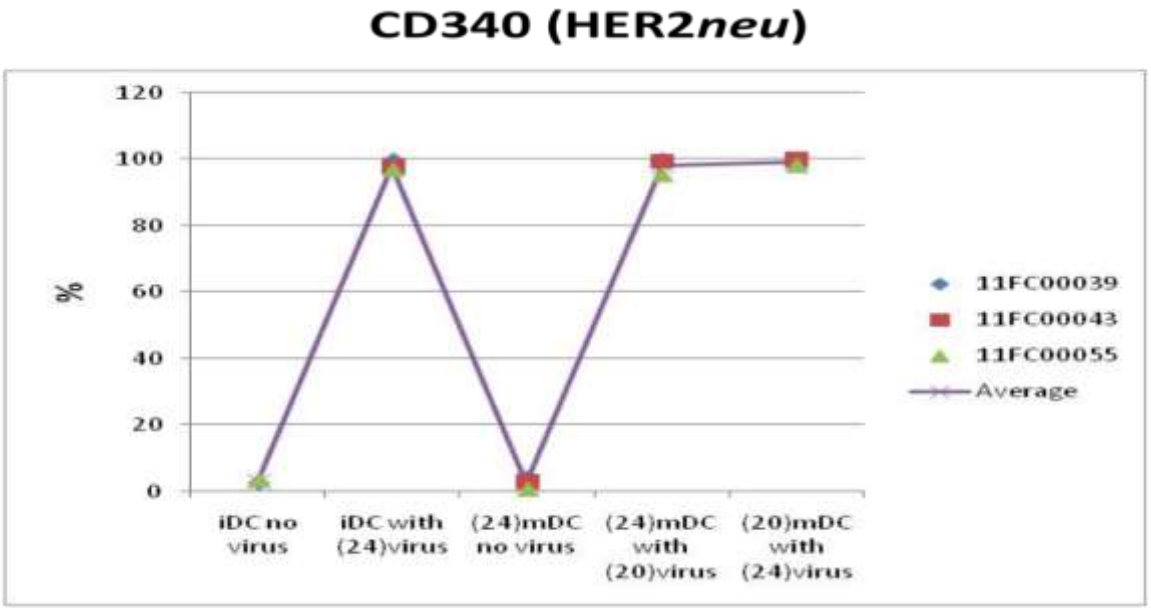


As depicted in **Figure 8**, the post transfection viability of matured DCs (mDCs) ranged from 60-82% (mean 70-75%). ***Since this post maturation/transfection DC viability is donor dependent and highly variable, we requested and received FDA approval allowing a release viability criteria of ≥ 60% rather than ≥ 70% for the final AdHER2 DC vaccine product to be used in this study.*** Historically, DTM reports that the product yield for transfected DC vaccine products is usually between 10-40% of the starting monocyte population. Note in **Figure 9** for an unknown reason, healthy donor 11FC00055 had an extremely poor yield of DCs post maturation/transfection. We have observed similar variations in vaccine viability and yields within and between donors in the manufacture of autologous TARP peptide DC vaccines on NCI protocol 09-C-0139.

HER2*neu* expression was also assessed under the different maturation conditions. There was significant variability of expression within and between donors under the different maturation and transfection conditions and immature DCs were observed to have greater variable expression of HER2*neu* i.e. the mean fluorescent intensity per cell of HER2 expression was more inconsistent on immature DCs *versus* mature DCs (personal communication L. Castiello and M. Sabatino of DTM, data not shown).

However, as documented below in **Figure 10**, ***HER2neu expression (CD340) was 100% on transfected immature and mature DCs, regardless of transfection conditions.***

Figure 10: DC HER2*neu* Expression Post Transfection/Maturation



Additional characterizations and assessments were also performed as part of these DC maturation/transfection experiments in an attempt to better characterize the qualitative, functional aspects of the final HER2*neu* DC vaccine product, including:

- Cluster of Differentiation (CD) antigens on the final DC vaccine product (in order of relative scientific priority): CD86, CD83, HLA-DR, CD38, CD54, CD14, and CCR7 (data not shown).
- Expression of cytokines and chemokines IL-6, IL-8, IL-12p70, TNF-α, IP10, MDC and MIP3β (expressed as a ratio that normalizes expression for the number of cells). Interferon-α, IL-10, IL-13 and IL-15 were at very low concentrations, usually < 5pg/mL (data not shown). Proteomic analysis of dendritic cell supernatants under the different transfection/maturation conditions is in process for the cytokines and chemokines mentioned above.

Based on these studies, we are planning to manufacture AdHER2 DC vaccine for this study using a 4 hour transfection of the AdHER2 vector <u>first</u> followed by the DC maturation cocktail (denoted as (20)mDC with (24) virus in the previous figures).

This decision is based on the following theoretical grounds as well as the observations from the *in vitro* studies documented above:

- It is generally easier to transfect immature DCs i.e. before maturation cocktail is added and ensure optimal antigen processing.
- There was more consistent HER2*neu* expression on mature DCs compared to immature DCs as measured by MFI, which may lead to greater uniformity of the final product.

The Certificate of Analysis for the final manufactured AdHER2 DC vaccine product will include documentation on the percentage of HER2neu expressing cells (%HER2*neu*) in the product and the HER2*neu* Mean Fluorescent Intensity (HER2*neu* MFI) of cells in the product to permit

preliminary assessment of their correlation with clinical outcomes. ***The above outlined transfection/maturation process for AdHER2 DC vaccine manufacture was approved by the FDA following their review of the data during the Pre-IND meeting held September 6th, 2011.***

1.2.7 Human Experience with Dendritic Cell Vaccines

There are a wide variety of platforms and immunogens that have been extensively studied in therapeutic cancer vaccine platforms including: a) whole tumor cell vaccines, b) dendritic cell (DC)-based platforms, c) peptide and fusion proteins co-delivered with adjuvants and c) viral vectors that serve as delivery vehicles for tumor-associated antigens (TAAs). In addition to classic peptide and protein immunogens, tumor lysates, tumor mRNA, and DNA have all been utilized. DCs serve as a bridge between the innate and adaptive immune system and in turn play a critical role in the activation of naïve CD4+ and CD8+ T cells. As a consequence, researchers in cancer immunotherapy have focused a tremendous amount of investigative effort to delineate and understand DC biology, activation, maturation, and antigen presentation. Because of the pervasive dysfunction of DCs *in vivo* as a consequence of the immune dysregulation associated with high tumor burdens[25], they have been the platform of choice for delivery of many tumor cell lines (LNCaP, PC3), peptides, proteins, lysates, mRNAs and viral vectors expressing TAAs[26,27]. Sipuleucel-T is a prototype of this platform approach: even though it is labeled as an *autologous cellular immunotherapy*, its generation utilizes similar maturation agents and pathways common to dendritic cell vaccines[28].

A better understanding of basic immunologic principles has led to a variety of techniques for enhancing tumor-specific immunity and their potential subsequent translation into improved clinical outcomes. This is exemplified by the FDA approval on April 29th, 2010 of sipuleucel-T (Provenge™), the first licensed therapeutic cellular immunotherapy and a major milestone in the field of cancer immunotherapy. Importantly, approval of sipuleucel-T was based upon a statistically persuasive and clinically meaningful 4.1-month *improvement in median overall survival (OS)* in the IMPACT phase III trial[29]. The improvement in OS associated with sipuleucel-T has been reported to correlate with CD54 upregulation, a measure of the product's potency[30] as well as the development of antibody titers exceeding 400 at any time against the immunizing antigen PA2024 (a fusion protein) or prostatic acid phosphatase (PAP)[29]. Interestingly, although very strong T cell proliferative responses to PA2024 and PAP were also observed in patients receiving sipuleucel-T, no difference or association in survival was documented between patients who exhibited T cell response to either antigen and those who did not.

DC vaccine products have been delivered intravenously (as is sipuleucel-T), intramuscularly, intranodally, intradermally and subcutaneously. In our pre-clinical model of rodent Ad*neu* ECTM DC vaccination, the vaccine was given subcutaneously. We are planning to deliver the human autologous HER2 DC vaccine equivalent used in this study intradermally, since it is in keeping with this model and there is evidence to suggest that in humans intradermal delivery may be more optimal than subcutaneous delivery (C. Mackall, personal communication).

1.2.8 Human Experience with HER2 Therapeutic Vaccines

The clinical efficacy of trastuzumab-based anti-HER2 passive immunotherapy and other HER2-targeted therapy has led to the development of vaccination strategies against HER2 that would ideally result in strong immunity producing immune memory associated with anti-tumor activity as well as prevention of tumor recurrence. Different anti-HER2 vaccine strategies currently

under investigation and in various stages of clinical development include DNA, peptide, whole tumor cell, and dendritic cell vaccines and combinations of the same e.g. peptide-loaded DC vaccines[31]. Peptide vaccines have included monovalent and as well as polyvalent HER2 peptides and been associated with the development of humoral as well as cellular immune responses. We believe the use of our proposed AdHER2 DC vaccine will induce polyclonal antibody responses directed towards both the extracellular (EC) and transmembrane (TM) components of HER2 as well as potentially the intracellular domain (IC) as a result of epitope spreading. The multiple, unique HER2 epitopes targeted by this autologous vaccine platform are likely to differ from trastuzumab binding epitopes and may result in greater affinity and clinical activity than that seen with trastuzumab, especially in patients with lower levels of HER2 expression i.e. <3+ by IHC. In addition, based on our pre-clinical data, the anti-HER2 antibodies induced by vaccination appear to have functional activity that, unlike trastuzumab, is *Fc receptor independent* and that interfere with HER2 expression and phosphorylation. Hence, vaccine-induced anti-HER2 antibodies may also have clinically relevant anti-tumor activity in patients with resistance to trastuzumab, lapitinib or other HER2-directed therapies.

1.2.9 Previous Human Experience with an Extracellular/Transmembrane/Intracellular HER2/neu Vaccine

Our collaborator Rolf Kiessling, and his colleagues at the Karolinska Institute in Sweden have conducted a pilot clinical trial of a plasmid DNA human HER2/*neu* vaccine encoding the extracellular (EC), transmembrane (TM) and mutated intracellular ($IC_{mutated}$) domains ($ECTMIC_{mutated}$) given with GM-CSF and IL-2[32]. As a safety measure, a two base pair mutation was introduced in the IC domain to remove an autophosphorylation site that confers oncogenicity and maximize safety. The vaccine was administered to a total of eight patients and no clinical issues of acute toxicity, autoimmunity or cardio toxicity were identified. As might be expected with a DNA plasmid construct, the vaccine demonstrated limited immunogenicity: no specific T-cell proliferation following *in vitro* stimulation of *ex vivo* PBMCs with recombinant human HER2 protein was induced by vaccination. In fact, immediately following three complete cycles of vaccination, no or even decreased HER2-specific CD4+ T-cell responses were observed, but a significant increase in MHC class II restricted HER2-T-cell responses was detected at long term follow-up. Because concurrent trastuzumab therapy was permitted during the study, λ-subclass specific ELISAs were performed to measure endogenous antibody production without trastuzumab interference. Importantly, in a subgroup of patients, HER2-pDNA vaccination induced and boosted HER2-specific antibodies that could be detected for several years following the last vaccine administration.

We propose to carry out this phase I and pilot study of vaccination with AdHER2 transduced autologous dendritic cells in patients with 1+ to 3+ HER2neu expressing tumors. Part I involves vaccine dose escalation in a *population with* ***no prior exposure*** *to trastuzumab or other HER2-directed agents* to determine if there is a significant, adverse safety signal regarding cardiac toxicity, in addition to preliminary assessment of the vaccine's immunogenicity and clinical activity. Five doses of 5, 10, 20 or 40 x10^6 viable cells/AdHER2 vaccine will be given intradermally at Weeks 0, 4, 8, 16 and 24 in patients with metastatic solid tumors and adjuvant bladder cancer patients characterized by some HER2/*neu* expression. ***While these patients may be less likely to respond due to lower expression levels of the HER2 target, it is equally possible that they may respond better because they will have less peripheral tolerance to HER2.*** In addition, AdHER2 DC vaccination is likely to induce a polyclonal antibody response

to multiple HER2 epitopes that differ from the EC epitope targeted by trastuzumab. These differing polyclonal, multiple-epitope responses may be associated with anti-tumor activity. Once preliminary cardiac safety is demonstrated, Part II of the study will repeat the vaccine dose escalation *in a population with* ***prior exposure*** *to trastuzumab and other HER2 directed therapies* (as required by the FDA) to again determine whether there is an adverse safety signal regarding cardiac toxicity, in addition to assessment of the vaccine's immunogenicity and clinical activity. Vaccine dose escalation, administration and re-staging assessment is identical to that conducted in Part I.

1.2.10 Exploratory Correlative Studies

1.2.10.1 Anti-HER2 Antibody Detection and Characterization

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1.2.10.2 JPT Peptide Technologies, Midissia Therapeutics and NCI

A major objective of this study is to determine the immunogenicity of the AdHER2 DC vaccine. Specifically, we seek to examine whether AdHER2 DC vaccination is associated with a 3-fold increase in anti-HER2/neu antibody concentration (measured as mcg/mL) or a 4-fold increase in antibody dilution titers over baseline. Overlapping HER2 peptide microarrays will also be utilized to characterize both vaccine-induced anti-HER2 antibody and cellular responses.

Anti-HER2 cellular response and clinical response relationship with anti-Ad5 Ab.

1.2.10.3 Anti-HER2 Cellular Response

The Berzofsky lab, through support from the NCI Frederick Clinical Support Laboratory will assess vaccine-induced HER2-specific responses as measured by IFN-γ, Granzyme A and Granzyme B ELISPOT assay and/or intracellular cytokine staining (ICS). The Berzofsky lab, through support from the NCI Frederick Clinical Support Laboratory will check humoral immunity i.e. anti-Ad5 antibody titers at baseline as well as post vaccination, to determine if autologous AdHER2 DC vaccination results in a change in these anti-Ad5 titers over time, or is correlated with the increases in anti-HER2 antibody titers post vaccination.

1.2.10.4 Circulating Tumor Cells (CTCs): Jane Trepel, DTB, CCR, NCI

Analysis will be performed by the Trepel laboratory utilizing ferrofluidic enrichment and multiparameter flow cytometric detection to study CTCs from a broad range of cancer types. CTCs will be identified as viable, nucleated cells, that positively express one or more epithelial or tumor markers and are negative for expression of hematopoietic markers. CTCs will be further characterized for markers for ICD such as calreticulin, Hsp70 and Hsp90.

1.2.10.5 Immune cell subsets: Jane Trepel, DTB, CCR, NCI

Peripheral blood mononuclear cells (PBMC) will be isolated from patient blood before and after treatment, labeled with a unique 2D barcode and viably stored. it will be thawed and assessed using multiparameter flow cytometry for immune subsets including but not necessarily limited to Tregs, myeloid-derived suppressor cells (MDSC), NK, NKT, CD4+ and CD8+ T-cells, and CD14+ monocyte subsets. Assessment will include functional markers, i.e. PD-1, Tim-3, CTLA-4, CD40, HLA-DR and PD-L1.

1.2.10.6 Function-Associated mRNAs in Whole Blood: Dr. Cindy Yamamoto, Hitachi Chemical

Although there have been major recent advances in the field of cancer immunotherapy with the approval of sipuleucel-T (Provenge®) and ipilimumab (YERVOY™), a significant challenge that remains is identification of immunologic responses that correlate with beneficial clinical outcomes such as tumor regression or survival. In collaboration with Dr. Cindy Yamamoto of Hitachi chemical, we will seek to explore whether characterization of a patient's baseline immune status using assessment of functional mRNAs can be utilized to predict who will respond to vaccination.

The assay is a novel high throughput real time PCR system for quantitatively assaying mRNA for any gene that is activated in cells[33]. Specifically, the methodology allows for quantification of 17 different leukocyte-function–associated mRNAs in whole blood after *ex vivo* stimulation with up to 8 different antigens (e.g. rIL2, PHA, Anti-TCR, HER2, heat aggregated IgG (HAG, immune complex to activate Fc receptor), LPS, Zymosan, and PBS control) on pre-manufactured strips. Since the volume blood needed for this test is very small and cell isolation and culture are not necessary, this exploratory methodology may serve as a model of personalized medical diagnostics for patient-specific cancer vaccine immunotherapy.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Common Eligibility for Parts I and II

2.1.1.1 Adults ≥ 18 with malignant soft tissue and bone tumors and recurrent or progressive, metastatic solid tumors who have progressed on standard therapies except in adjuvant for high risk bladder cancer in Part I.

2.1.1.2 Recurrent or progressive disease on prior standard therapies with known clinical benefit with the exception of adjuvant bladder cancer population.

2.1.1.3 Performance Status: ECOG 0-1. (Refer to Appendix 1)

2.1.1.4 Baseline LVEF by 2D Echocardiogram ≥ 53%.

2.1.1.5 Greater than or equal to 1 week since standard or investigational treatment for metastatic disease.

2.1.1.6 Stable, concurrent use of hormone therapy for hormone receptor positive breast cancer is permitted.

2.1.1.7 Hematologic parameters: ANC ≥ 1000 cells/mm3, ALC ≥ 300 cells/mm3, Hemoglobin ≥ 9.0 gm/dL, WBC ≥ 2,500 cells/mm3, platelet count ≥ 75,000/mm3, PT/PTT ≤1.5X the upper limits of normal.

2.1.1.8 Chemistry parameters: Creatinine ≤ 1.5 mg/dL, SGOT and SGPT ≤ 3X the upper limits of normal and total bilirubin ≤ 1.5 mg/dl, Alk PO4 ≤3X the upper limits of normal (except for patients with documented metastatic disease to bone and/or liver).

2.1.1.9 Negative serum beta HCG if female and of childbearing potential.

2.1.1.10 Negative HIV 1/2 serology and sample drawn for HTLV. Patients with HIV are excluded from participating on this clinical trial because their immunodeficiency would confound the evaluation of adverse events which would hinder meeting the primary objective.

2.1.1.11 Negative serology for hepatitis B and C unless the result is consistent with prior vaccination or prior infection with full recovery.

2.1.1.12 Willingness of female and male subjects to use effective contraception e.g. oral contraceptives, barrier device, intrauterine device, or condoms, during the study and for three months following the last dose of study vaccine. We suggest that subjects do not become pregnant or father a child during the study, and for 3 months following receipt of the investigational AdHER2 DC vaccine.

2.1.1.13 Able to understand and provide Informed Consent.

2.1.1.14 Patients with 1+ to 3+ HER2/neu expression by IHC or an equivocal or positive FISH result by 2013 ASCO/CAP guideline.

HER2 Detection Method	**Negative**	**Equivocal**	**Positive**
Vysis FISH Ratio (Measures HER2 oncogene)	-Single-probe average HER2 copy number <4.0 signals/cell -Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell	-Single-probe ISH average HER2 copy number ≥4.0 and <6.0 signals/cell -Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell	-Single-probe average HER2 copy number ≥ 6.0 signals/cell -Dual-probe HER2/CEP17 ratio ≥ 2.0 with an average HER2 copy number <4.0 signals per cell -Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number <4.0 signals/cell -Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥6.0 signals/cell
IHC (Measures HER2 protein)	0-1+	2+	3+

2.1.1.15 Patients must have measurable disease, per RECIST 1.1. See Section **6.2** for the evaluation of measurable disease except adjuvant high risk bladder cancer.

2.1.2 Part I Eligibility

2.1.2.1 Naïve to trastuzumab (Herceptin™), pertuzumab (Perjeta™) and lapatinib (Tykerb™), ado-trastuzumab emtansine (Kadcyla™) or other HER2-directed therapies.

2.1.2.2 Malignancy as follows:

- Malignant soft tissue and bone tumors and recurrent or progressive, metastatic solid tumors who have progressed on standard therapies; or,
- Bladder cancer in the adjuvant setting (adjuvant bladder cancer patients):
  - Tumor stage T3a, T3b, T4a, T4b and any node positive disease regardless of tumor stage.
  - Status-post primary cystectomy with curative intent.
  - May or may not have received neoadjuvant cisplatin-based combination chemotherapy per NCCN guidelines.
  - May or may not have received adjuvant radiotherapy or chemotherapy based on pathologic risk per NCCN guidelines.
  - Greater than or equal to 6 weeks s/p primary surgery with curative intent.
- NOTE: Patients with breast, ovarian, cervical, colon, gastric/gastroesophageal junction, non-small cell lung, renal cell, bladder, malignant soft tissue and bone tumor, prostate cancer or other solid tumors.

2.1.3 Part II Eligibility

2.1.3.1 Malignant soft tissue and bone tumors and recurrent or progressive, metastatic solid tumors who have progressed on standard therapies.

2.1.3.2 Recurrent or progressive metastatic disease after standard of care HER2-targeted therapies; i.e. trastuzumab (Herceptin™), pertuzumab (Perjeta™), lapatinib (Tykerb™), ado-trastuzumab emtansine (TDM1) (Kadcyla™) or other HER2-directed therapies.

2.1.3.3 Stable, concurrent use of tamoxifen or aromatase inhibitors for hormone receptor positive breast cancer is permitted.

2.2 EXCLUSION CRITERIA

2.2.1.1 Pregnant women are excluded from this study because AdHER DC vaccine may have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with AdHER DC vaccine, breastfeeding should be discontinued if the mother is treated with AdHER DC vaccine.

2.2.1.2 Patients with active CNS metastases or leptomeningeal involvement by tumor (patients with a history of brain metastases who have successfully treated for brain metastasis by surgery or radiation and who have not had any evidence of the new or progressive CNS disease for more than 12 months are eligible).

2.2.1.3 Patients with rapidly progressing disease in the opinion of the Principal Investigator.

2.2.1.4 Patients with inadequate bilateral peripheral venous or central venous catheter access for the required apheresis to allow generation of the autologous AdHER2 DC vaccine product.

2.2.1.5 Clinically significant cardiac dysfunction defined as a history of ≥ NYHA Class II symptoms, angina, congestive heart failure, myocardial infarction, arrhythmias or cardiac dysfunction requiring treatment or discontinuation of chemotherapy.

2.2.1.6 History of changes in baseline LVEF that occurred during prior treatment with anti-HER2 treatment.

2.2.1.7 Cumulative doxorubicin dose ≥ 400mg/m^2 (>450 mg/m^2 for malignant soft tissue and bone tumor patients) or cumulative epirubicin dose ≥ 800mg/m^2.

2.2.1.8 Use of any standard chemotherapy or other investigational agent(s) within 1 week of study enrollment.

2.2.1.9 Use of systemic corticosteroid therapy within 2 weeks of study enrollment, including patients receiving replacement corticosteroid therapy. Note: *only* topical, inhaled and intranasal steroid therapy is permitted.

2.2.1.10 Active systemic viral, bacterial or fungal infection requiring treatment.

2.2.1.11 A medical history which the treating physician believes causes the patient to be excluded. This includes a remote history of cancer. Please note: squamous cell carcinoma, basal cell carcinoma and remote history of cancer with no evidence of recurrence for the past 5 years are eligible.

2.2.2 Recruitment Strategies

This protocol is being conducted in collaboration with Dr. Henriette Hansen of JPT Peptide Technologies as well as Dr. John Morris of the University of Cincinnati, who was also significantly involved in the pre-clinical animal model studies of the AdHER2 vaccine platform before he left the NCI. We anticipate that a significant number of HER2 positive patients will be referred from these colleagues in addition to patients identified through affiliates at Walter Reed National Military Medical Center. We will submit approved protocol to Dr. Susan Love Research Foundation's Army of Women Program (www.armyofwomen.org/) and ResearchMatch (https://www.researchmatch.org) for review to allow contact with and recruitment through their volunteers. We also made contact with Office of Patient Recruitment, National Institutes of Health Clinical Center to update our contact information, generate materials including flyers, posting for social media and posters. Additionally, this study will be posted on NIH websites and on NIH social media forums. NCI Vaccine Branch Clinical Trials team also opened a twitter account (@NCICCR_VB) for the patient recruitment and sharing the updated information on cancer vaccine and related topics with other professionals and general public. This account is officially registered to Office of Communications and Public Liaison at NCI.

In addition to the standard electronic mailings and postings by the NCI CCR Office of Patient Outreach and Recruitment, the former Principal Investigator, now a special volunteer at Vaccine Branch, CCR, NCI, Dr. Wood developed a brief video (no more than 5 minutes in length) that provides an informational overview to both patients and health care providers describing the scientific rationale for and design of the clinical study.

This video was posted to the YouTube channels of the NCI Office of Communications and the NIH Clinical Center. Given that so much of consumer media information is now provided in video format that can easily be accessed via the web using computers and smart phone operating systems and applications platforms, we believe that this could serve as a novel recruitment tool

for patients interested in participating in clinical research studies. See **Appendix 13**: Patient Recruitment Materials.

2.3 PRE-SCREENING AND SCREENING EVALUATION

2.3.1 Pre-Screening

All potential patients must provide written documentation of the HER2 status of their primary tumor and/or a recent tumor biopsy specimen, along with availability of a paraffin tissue block. For those with incomplete HER2 characterization as defined by the IHC or FISH, HER2 characterization will be performed by the NCI Laboratory of Pathology.

2.3.2 Screening Evaluation

Patients will undergo a medical history and physical examination, review of concomitant medications, including review of symptoms, performance status, and life expectancy and apheresis clinic access assessment at screening. History will also specifically document:

- Prior exposure to anthracycline chemotherapy agents and total cumulative dose.
- Prior exposure to HER2-directed therapies: agent, duration and number of rounds of treatment.
- Documentation of New York Heart Association (NYHA) functional classification. (Refer to **Appendix 2**)
- Imaging studies (obtained within 30 days prior to enrollment). Note: if the patient had scans at an outside institution the patient will not be required a screening scan to consent. Instead, the baseline scan will be performed at NIH prior to treatment and used to identify target lesions.
    - CT scan of neck (optional), chest, abdomen and pelvis
    - MRI of the brain (in select clinically indicated patients)
    - Technetium 99 bone scan (if clinically indicated, for patients with bone metastases)
- 2D Echocardiogram with documentation of LVEF (obtained within 30 days prior to enrollment). Note: if the patient had an echo at an outside institution the patient will not be required a screening echo to consent. Instead, the baseline echo will be performed at NIH prior to treatment)
- Urinalysis
- Laboratory evaluation (obtained within 30 days prior to enrollment)
- CBC with differential counts, PT/PTT, acute care panel, hepatic panel, serum beta-HCG (for females with childbearing potential only), anti-HIV-1/2 Ab, anti-HCV Ab, HBsAg, HBs Ab, anti-HTLV-1/2 Ab, anti-CMV IgG/IgM and RPR

Refer to Section **3.4** for the Study Calendar schedule of on study clinical, laboratory, radiographic and research evaluations.

2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team.

2.4.1 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Solid Tumors HER2-directed therapies naive dose escalation cohort	Patients with ovarian, cervical, colon, non-small cell lung, renal cell, bladder, malignant soft tissue and bone tumor, prostate cancer or other solid tumors as well patients with breast cancer to be treated with AdHER DC vaccine at escalating doses.
2	Solid Tumors HER2-directed therapies naïve expansion cohort	Patients with ovarian, cervical, colon, non-small cell lung, renal cell, bladder, malignant soft tissue and bone tumor, prostate cancer or other solid tumors as well patients with breast cancer to be treated with AdHER DC vaccine at 40x10e6 dendric cells maximum.
3	Solid Tumors with prior HER2-directed therapies dose escalation cohort	Patients with recurrent or progressive, metastatic breast cancer, gastric/gastroesophageal or other cancers treated with AdHER DC vaccine at Dose Level 1.
4	Solid Tumors with prior HER2-directed therapies expansion cohort	Patients with recurrent or progressive, metastatic breast cancer, gastric/gastroesophageal or other cancers enrolled in dose expansion cohort.

Arms

Number	Name	Description
1	Part I dose escalation	AdHER DC vaccine administered at escalating doses.
2	Part I dose expansion	AdHER DC vaccine administered at 40x10e6 dendric cells maximum.
3	Part II dose escalation	AdHER DC vaccine administered at Dose Level 1.
4	Part II dose expansion	AdHER DC vaccine administered at 40x10e6 dendric cells maximum.

Randomization and Arm Assignment

There will be no randomization on this study.

Up to 18 patients in Cohort 1 will be assigned to Arm 1

Up to 12 patients in Cohort 2 will be assigned to Arm 2

Up to 6 patients in Cohort 3 will be assigned to Arm 3

Up to 24 patients in Cohort 4 will be assigned to Arm 4

2.5 BASELINE EVALUATION

Below studies will be obtained within 30 days prior to the administration of the first dose of vaccine. If any of the studies were performed on another protocol, they may not need to be repeated.

- CBC with Differential
- Acute care panel, hepatic panel, mineral panel, total protein, amylase, lipase, uric acid
- PT/PTT
- Lipid panel
- Immunoglobulin quantification, IgE
- LDH
- CPK, Troponin I
- Thyroid screen (TSH, Free T4)
- 25-OH-vitamin D
- Lymphocyte subsets
- Tumor markers (optional)
- Urinalysis
- ABO typing and HLA A, B, C and DR (if not done in NIH Clinical Center in the past)
- CT CAP (to be done only if screening scans were performed at an outside institution and not repeated at the NCI).
- MRI Brain (to be done only if clinically indicated and screening scans were performed at an outside institution and not repeated at the NCI).
- CT Neck (to be done only if clinically indicated and screening scans were performed at an outside institution and not repeated at the NCI).
- Bone Scan (to be done only if patient has bone metastasis and screening scans were performed at an outside institution and not repeated at the NCI).
- Echocardiogram (to be done only if screening echo(s) were performed at an outside institution and not repeated at the NCI).

3 STUDY IMPLEMENTATION

3.1 STUDY SCHEMA AND DESIGN

This is an open label, single site, non-randomized, two-part, phase I study of an autologous dendritic cell (DC) vaccine transduced with AdHER2 to determine vaccine safety, immunogenicity and preliminary activity. The study design schema is outlined in **Figure 11**.

Once enrollment eligibility is confirmed and informed consent is obtained, the patient will be scheduled for an apheresis of up to 18 liters to collect mononuclear cells by counter-flow elutriation, aliquoted into minimum of 6 vials with up to 333 x 10^6 cells/vial that will be cryopreserved for future preparation of the autologous AdHER2 DC vaccine product. If the collected apheresis products are not adequate for 5 doses of vaccine, the patient may undergo

additional apheresis per investigator's discretion. Co-enrollment in the ^{111}Indium trastuzumab imaging protocol 07-C-0101 will be offered to those patients that are interested and eligible.

Note: In order to be cleared for the next treatment, a safety visit (i.e., physical and clinical laboratory assessments, review of toxicities/adverse events) must occur within 7 days prior to the treatment. Treatment visits can also be safety visits for previous injections. This is so the treating physician will evaluate the patient for toxicities potentially caused by previous injections.

Part I N = 30 patients

Part I of the study will investigate vaccine dose escalation in adults with recurrent or progressive, metastatic solid tumors as well as adjuvant bladder cancer patients whose tumors are characterized by some HER2/*neu* expression but for whom trastuzumab is *not* clinically indicated:

- Patients with ovarian, cervical, colon, non-small cell lung, renal cell, bladder, malignant soft tissue and bone tumor, prostate cancer or other solid tumors that is known to be HER2 1+, 2+ or 3+ by IHC OR have a Vysis FISH result ≥ 1.8.
- Patients with breast cancer that is known to be HER2 2+ or 3+ or with an equivocal or positive FISH result.

NOTE: Adjuvant bladder cancer patients will not be enrolled in Part I of the study until AdHER2 DC vaccine safety has been demonstrated out to 12 Weeks in at least 10 patients.

The goal of Part I is to determine whether there is a preliminary significant, adverse safety signal regarding cardiac toxicity in this patient population that is *naive to HER2-directed therapies*, in addition to preliminary assessment of the vaccine's immunogenicity and clinical activity. Allowable tumors include breast, ovarian, colon, non-small cell lung, renal cell, bladder and prostate cancer. Inclusion of a broad spectrum of tumors that express HER2 will facilitate more rapid accrual and allow prompt determination of the vaccine's safety and immunogenicity. However, the inclusion of subjects with multiple tumor types that have varying levels of HER2 expression may preclude identification of an optimal biologically active dose.

Proposed Dosage and Administration

This part of the study will enroll patients in the vaccine dose levels outlined below:

Dose Level 1 (N = 6 patients):	5 x 10^6 *viable* cells/vaccine
Dose Level 2 (N = 6 patients):	10 x 10^6 *viable* cells/vaccine
Dose Level 3 (N = 6 patients):	20 x 10^6 *viable* cells/vaccine
Expansion Cohort (N = 12 patients):	40 x 10^6 *viable* cells/vaccine

Autologous AdHER2 DC vaccine will be administered intradermally at Weeks 0, 4, 8, 16 and 24. Response will be measured as outlined in section **6.2**. Vaccine dose escalation will follow the procedures specified in Section **3.1.2**.

Part II N = 30 patients

Part II of the study will be conducted in adults with recurrent or progressive, metastatic breast cancer, gastric/gastroesophageal or other cancers with 1+ to 3+ HER2/neu expression by IHC or an equivocal or positive FISH result. Vaccine dose escalation (as required by the FDA) will be repeated in an identical manner *in a population with **prior exposure** to trastuzumab, pertuzumab, lapatinib, ado-trastuzumab emtansine (TDM1) and other HER2-directed therapies)* to again determine whether there is an adverse safety signal regarding cardiac toxicity, in addition to assessment of the vaccine's immunogenicity and clinical activity. Vaccine administration and

re-staging assessment is identical to that conducted in Part I with revised vaccine dosing as outlined below and in Section **3.1.2**.

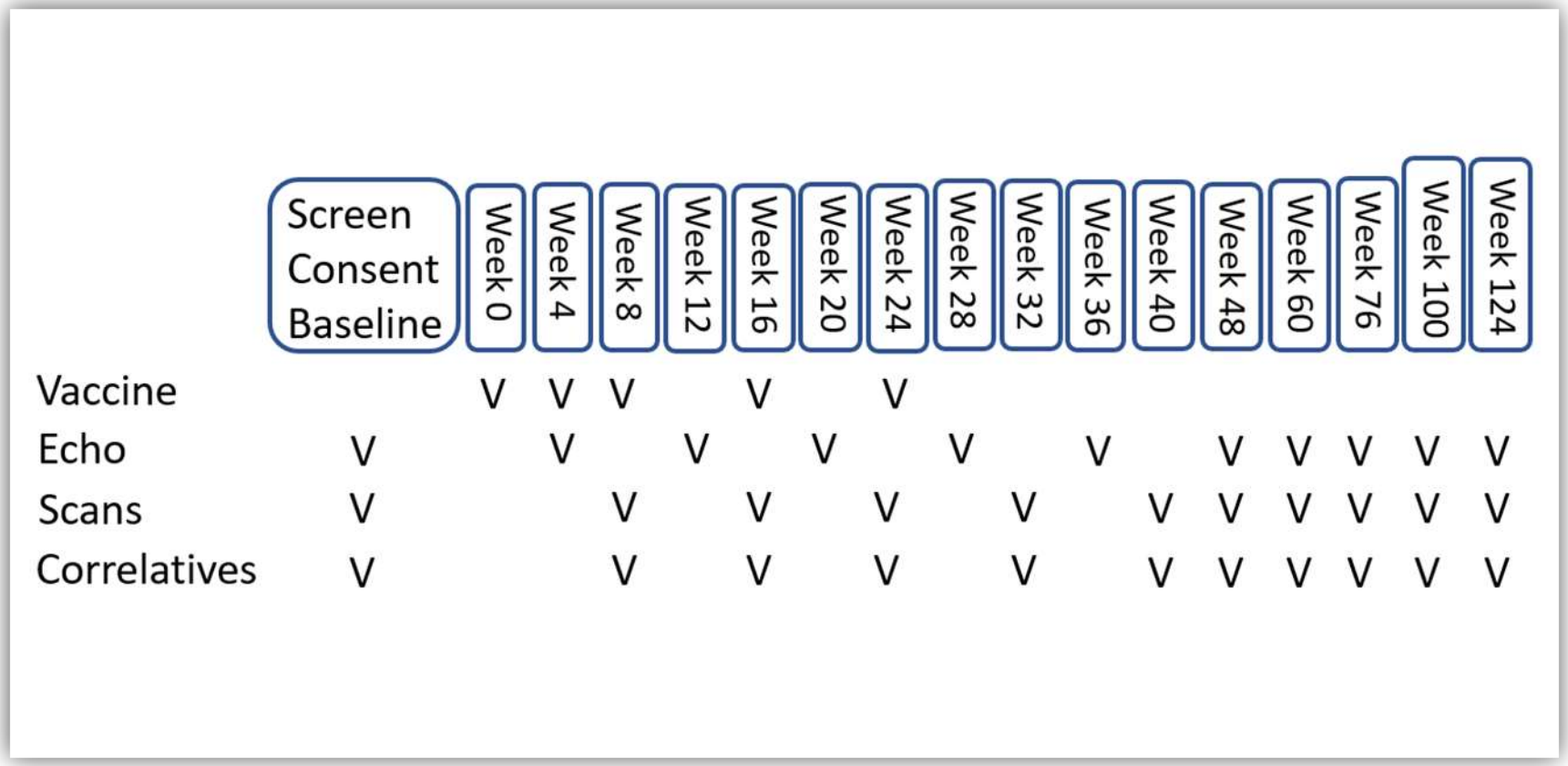
Proposed Dosage and Administration

Part II of the study will enroll 30 breast, gastric/gastroesophageal or other cancer patients as outlined in the vaccine dose cohorts outlined below:

Dose Level 1 (N = 6 patients):	20 x 10^6 *viable* cells/vaccine
Expansion Cohort (N = 24 patients):	40 x 10^6 *viable* cells/vaccine

Autologous AdHER2 DC vaccine will be administered intradermally at Weeks 0, 4, 8, 16 and 24. Re-staging will be performed as outlined in Section **6.2**. Vaccine dose escalation will follow the revised procedures specified in Section **3.1.2**.

Figure 11: AdHER2 Autologous DC Vaccine Study Schema



3.1.1 Dose Limiting Toxicity

The following assessment guidelines for the management of dose limiting toxicity (DLT) are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE and DLT reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

All adverse events will be classified as follows for determination of relatedness to AdHER2 vaccination:

- Unrelated
- Unlikely
- Possibly related
- Probably related
- Definitely related

For the purposes of this study, the definitions of dose limiting-toxicities include:

- CTCAE Grade 2 and greater allergic reactions
- CTCAE Grade 2 and greater autoimmune disorders
- CTRCD, defined as a decrease in the LVEF of > 10 percentage points, to a value in < 53%[1]. Documentation of the above specified change in LVEF will require cardiac consultation for determination of clinical significance. Cardiac function deterioration clinically associated with apparent cardiotoxic agent use after off-therapy will be censored at the point of cardiotoxic agent use.
- CTCAE Grade 3 or greater organ toxicity (dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) occurring within 30 days of vaccine administration that are at least possibly related to research in.

Immunization-Related Dose Limiting Toxicity:

- CTCAE Grade 3 and greater anaphylaxis: symptomatic bronchospasm with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedma; hypotension (Grade 3). Life threatening consequences; urgent intervention indicated (Grade 4).
- CTCAE Grade 3 and greater injection site reactions: ulceration or necrosis; severe tissue damage; operative intervention indicated (Grade 3). Life threatening consequences; urgent intervention indicated (Grade 4).

Early stopping rules for accrual will be invoked in each part of the study if:

- ***Two or more of 6 patients in a given dose level develop evidence of cardiac toxicity, accrual will be stopped to that dose cohort. Accrual expansion of an additional 12 patients will occur in the next lower dose level to further evaluate for cardiac toxicity.***
- ***Any death (unrelated to underlying primary disease) that occurs and is attributed as being possibly, probably or definitely related to the investigational AdHER2 DC vaccine (FDA requirement)***

3.1.2 Dose Escalation

It has been reported that up to 7% of patients with HER2/*neu* expressing tumors treated with trastuzumab monotherapy for metastatic breast cancer developed clinically significant cardiac dysfunction including congestive heart failure. It would be considered acceptable if the therapeutic AdHER2 vaccine proposed for this study would produce no greater than that level of cardiac toxicity, but not if it were to be exceeded. In order to evaluate this using a limited number of patients, a two-part design, but incorporating an early stopping rule, will be implemented. The goal is to accrue 30 evaluable patients in each of Part I and Part II of the study and treat them with the experimental AdHER2 vaccine. Unless an early stopping rule is invoked, the number of patients who have experienced a clinically significant cardiac toxicity will be determined to estimate the frequency of cardiac toxicity. In addition, the dose level(s) at which toxicity is observed will also be examined. The statistical rationale for the targeted accrual of 30 patients in each part of the study is outlined in the statistical Section **10**.

Dose escalation will proceed in groups of 6 patients. *Dose escalation will proceed as long as 0 to1 of 6 patients at given dose level have no evidence of toxicity and at least 3 of the 6 patients at the dose level have reached Week 12 on study.* If 2 of 6 patients on any given dose level develop cardiac toxicity, that dose cohort will be closed to further accrual, no higher doses will be explored, and dose expansion of an additional 12 patients will be enrolled in the next lower dose level to further assess for cardiac toxicity. *The vaccine dose at which 0 to 1 of 18 treated patients in Dose Levels 1 to 3 develop cardiac toxicity will be defined as the maximum tolerated dose (MTD). If two or more patients out of 18 at a given dose level have developed cardiac toxicity, then the MTD will have been exceeded at that dose, and the dose level below that one will be considered the MTD.* Since enrollment in Dose Level 3 has been reached and 0 of 6 patients has toxicity, then an expansion cohort of 12 patients, will be added for a maximum targeted accrual of 30 patients in Part 1. The 12 patients will be accrued at the 40x10^6 dose level without any planned interim stop, as cardiac toxicity is not presently anticipated given the results as of 12/11/15. With all 12 enrolled as a single group, there is a 4.9% probability of having 2 or more of 12 patients with cardiac toxicity if the true probability for a given patient is 3%, and 55.7% of having 2 or more of 12 with cardiac toxicity if the true probability for a given patient is 15%. Therefore, observing 0-1 of 12 with toxicity would be acceptable, and 40 x10^6 would be considered the maximum evaluated and tolerated dose. If we observe 2 or more with cardiac toxicity at or before accruing the 12th patient, this exceeds a safe level. Further patients would be added subsequently, by amendment, to the next lower dose, to confirm its safety.

If dose escalation has proceeded in the first three dose levels in Part I and 0 of 20 patients have experienced cardiac toxicity, Part II of the study in patients with metastatic breast, gastric, gastroesophageal junction or other cancers and prior exposure to HER2-directed therapies will proceed with vaccine dosing based on the safety confirmed in Part I and outlined in Section **3.1.2**. A maximum accrual of 30 patients will be enrolled in Part II.

If no DLTs are observed, then dose levels in Part I and Part II will be assessed for the fraction of patients with immunogenicity and clinical outcomes to determine the biologically optimal vaccine dose that would be used in any future phase II studies involving those respective patient populations.

To allow for the possibility of inevaluable patients, an accrual ceiling of 65 will be used.

3.2 DRUG ADMINISTRATION

All patients will undergo up to18-liter apheresis to remove peripheral blood monocytes for dendritic cell preparation. Cells used for subsequent dendritic cell maturation will be derived from monocytes frozen during the initial apheresis. Autologous AdHER2 dendritic cell vaccine will be manufactured under cGMP conditions by the NIH Clinical Center Department of Transfusion Medicine as outlined in **Appendix 3.** If the mononuclear cells or plasma collected are not adequate for minimum of 5 doses of vaccine, the patient will undergo optional additional leukapheresis or plasma collection per investigator's discretion.

- Autologous AdHER2 DC vaccine preparations will be assessed for release standards (nucleated cell content and concentration, appearance, flow cytometric verification of DC validation markers, viability ≥ 60%, and product sterility and safety testing) as well as for HER2 expression (percent of cells expressing HER2/*neu* and the ratio of geometric mean fluorescent intensity (MFI) of HER2/*neu* expression to isotype control).
- For patients in both Part I and Part II of the study, autologous AdHER2 DC vaccinations will be dosed according to enrollment dose levels (5, 10, 20, or 40 x 10^6 *viable* cells/vaccine) and administered intradermally in up to two injection sites at Weeks 0, 4, 8, 16 and 24 for a total of five vaccinations. Vaccine will be administered in the 3SE Day Hospital and patients will be monitored for immediate adverse event vaccine reactions (VS, clinical assessment) for 1 hour following their first HER2 DC vaccine dose. If no adverse reactions are observed with the first vaccination, patients will be monitored for 15 minutes for all subsequent vaccinations.
- If an adverse reaction is observed following the first vaccine, the reaction will be characterized and a determination made as to whether it is considered a dose limiting toxicity (DLT) as outlined in Section **3.1.1**. If the adverse reaction is determined *not* to be a DLT, the duration of post-vaccination monitoring for subsequent vaccinations will be determined by the Principal Investigator and Lead Associate Investigator as clinically indicated depending on the severity of the initial vaccine reaction.
- All patients will be given an AdHER2 DC Vaccine Report Card (refer to **Appendix 5**) and instructed on how to complete it, following each AdHER2 DC vaccine dose.
- Continued injection of the same forearm is permissible for patients who have one functional forearm or a medical condition that does not allow for continued injections of both forearms.

Since this protocol involves AdHER2 DC vaccination in humans using this vector for the first time, enrollment into subsequent dose level for both Part I and Part II patients will be staggered to allow for safety monitoring as outlined in Section **3.1.2**: *dose escalation will proceed as long as 0 of 6 or 1 of 6 patients within a dose level have no evidence of toxicity and at least 3 of the 6 patients within the same dose level have reached Week 12 on study* before enrollment in the next vaccine dose level can begin. If no dose limiting toxicities as defined in Section **3.1.1** are observed in the twelve-week window inclusive of the first three vaccinations, enrollment of additional patients may proceed as quickly as is logistically feasible as outlined above and in accordance with the dose escalation parameters outlined in Section **3.1.2**.

3.3 DOSE MODIFICATION AND IMMUNIZATION STOPPING RULES

Unlike trastuzumab, the AdHER2 vaccine being given in this study targets both the extracellular (EC) and transmembrane (TM) domains of HER2. Use of trastuzumab in women with breast cancer has been shown to be associated with a small, but significant risk (about 7%) for developing cardiac dysfunction. ***Exactly how trastuzumab causes cardiac toxicity is unknown.*** The goal of vaccination with this product is to stimulate the patient's own immune system to make antibodies (and also potentially killer cells) that recognize HER2. ***It is not known whether or not antibodies made by a person's own immune system will cause cardiac dysfunction.*** In addition, if antibodies made by the immune system following AdHER2 DC vaccination *were* to cause cardiac toxicity or other significant clinical adverse events, it would be very challenging because the immune system cannot simply be "turned off".

Thus, no dose modifications will be made in patients receiving AdHER2 DC vaccination. Subjects will cease to receive immunization if they experience dose-limiting toxicity (DLT) as outlined in Section **3.1.1**.

Supportive Management of Patients Experiencing DLT:

- For patients experiencing Grade 2 or greater allergic or autoimmune DLT, supportive clinical intervention will be provided until the DLT resolves to ≤ Grade 1.
- For patients experiencing Grade 2 or greater cardiac DLT, supportive clinical intervention will be provided until the DLT resolves to ≤ Grade 1.
  - Patients will also undergo repeat LVEF measurements at 4-week intervals until LVEF returns to baseline function. Thereafter, cardiac function will continue to be monitored according to the study schema outlined in **Figure 11** until study Week 124.
  - Patients who experience Grade 2 or greater cardiac DLT that does *not resolve* to ≤ Grade 1 will continue to receive clinical support and be monitored according to the study schema outlined in **Figure 11**.
  - until study Week 124.
- For patients experiencing Grade 3 or greater organ (dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary or neurologic) DLT supportive clinical intervention will be provided until the DLT resolves to ≤ Grade 1.
- For patients experiencing Grade 3 or greater anaphylaxis DLT, supportive clinical intervention will be provided until the DLT resolves to ≤ Grade 2.
- For patients experiencing Grade 3 or greater local injection site reactions, supportive clinical intervention will be provided until the DLT resolves to ≤ Grade 1.
- **NOTE:** Once patients have completed participation in this study, ***there is no provision for long term medical care*** by the Clinical Center, NIH or the NCI.

3.4 STUDY CALENDAR

Study Procedures	*Screen*	*Wk 0*	*Wk 4*	*Wk 8*	*Wk 12*	*Wk 16*	*Wk 20*	*Wk 24*	*Wk 28*	*Wk 32*	*Wk 36*	*Wk 40*	*Wk 48[17]*	*Wk 60[18]*	*Wk 76[18]*	*Wk 100[18]*	*Wk 124[18]*
NIH Advanced Directives[1]	√																
Informed Consent	√																
Height/Wt/NYHA	√																
Screening labs/HLA[2]	√																
Physical Exam/ECOG[3]	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Clinical labs[4]	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Tumor Markers[5]	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
LDH/CK/Troponin[6]	√	√	√	√	√	√	√	√	√	√	√						
TSH/25-OH Vit D	√	√						√					√				
Lymphocyte Subsets/ Ig QA/IgE	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Serum beta HCG[7]	√	√	√	√	√	√	√	√	√	√	√						
Urinalysis	√	√						√					√				
Anti-HER2 Ab/ Profile[8]		√		√		√		√		√		√	√	√	√	√	√
Anti-Ad5 Antibody[9]		√		√		√		√		√		√	√	√	√	√	√
Cellular HER2 Response[10]		√		√		√		√		√		√	√	√	√	√	√
Immune Subset/CTCs[11]		√		√		√		√		√		√	√	√	√	√	√
Hitachi mRNA		√															
MRI Brain[12], CT Neck[12], bone scan[13]	√			√		√		√		√		√	√	√	√	√	√
CT Chest/Abd/Pelvis[14]	√			√		√		√		√		√	√	√	√	√	√
2-D ECHO[15]	√		√		√		√		√		√		√	√	√	√	√
AdHER2 DC vaccine[16]	*Apheresis*	√	√	√		√		√									
Vaccine Report Card		√	√	√		√		√									
Adverse Events		√	√	√	√	√	√	√	√								

[1] As indicated in Section **12.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required

[2] Screening labs: CBC diff, PT/PTT, acute care panel, hepatic panel, serum beta HCG, urinalysis, and the following DTM labs: Anti-HIV 1/2 Ab, Anti-HCV Ab, HBsAg, Anti-HBs Ab, anti-HTLV 1/2 Ab (pending result is acceptable for eligibility), anti-CMV IgG/IgM, and RPR. If DTM labs are performed > 30 days prior to apheresis, DTM labs must be repeated. HLA will not determine eligibility and if it was done previously at the NIH it does not need to be repeated.

[3] Patients should be evaluated within 7 days prior to upcoming vaccination as a clearance for treatment.

[4] Clinical labs CBC with differential, PT/PTT, acute care panel, hepatic panel, mineral panel, total protein, amylase/lipase, uric acid, lipid panel.

[5] Tumor markers are optional and will be tested dependent on the primary sites. Patients with primary breast, ovarian and GI tract cancer might be tested for CA 125, CA 15-3, CA19-9, CA 27-29, and CEA. Other organ specific tumor markers can be tested

[6,7] Only until 12 weeks after the last dose of vaccination.

[8-11] Research bloods will not be used in data analysis if the patient has started on any treatment that can affect the data of the study. Week 40 and after samples will only be collected if the patient has not progressed (PD).

[12] This is optional and to be performed if clinically indicated. If the patient received scans within 30 days of enrollment at an outside institution the patient will not be required to have a second scan performed. Instead, the baseline scan will be used to collect target lesions.

[13] If the patient received scans within 30 days of enrollment at an outside institution the patient will not be required a screening scan to consent. Instead, the baseline scan will be used to identify target lesions. If the patient has bone metastasis and clinically indicated, a bone scan will be done at restaging.

[14] If the patient received scans within 30 days of enrollment at an outside institution the patient will not be required to have a second scan performed. Instead, the baseline scan will be used to collect target lesions. If a confirmatory scan is performed in less than 8 weeks from prior CT, the interval of CT follow-up will be adjusted to maintain 8 weeks interval to avoid unnecessary radiation exposure. For bladder cancer adjuvant indication, CT scans every 12 weeks during first 12 month, every 16 weeks for the next 12 months and at the end of study if no recurrence.

[15] Optional documentation on cardiac function will be collected once a patient is on study but off treatment for the purposes of long-term assessment of the impact of AdHER2 vaccination on cardiac function. Note: if the patient had an echo within 30 days of enrollment at an outside institution the patient will not be required a screening echo to consent. Instead, the baseline echo will be performed at NIH prior to treatment and used to identify target lesions.

[16] Dose can be delayed up to 8 weeks. The study will reset at the time of study resumption. For example, if dose at week 8 is delayed and resumed at original week 11, that week 11 will now be considered week 8.

[17] If there is a need for confirmatory scans based on the result of Week 48 evaluation, additional visit will occur at week 52 which will include clinical assessment with physical exam and laboratory assessment.

[18] Patients who progress do not require a full evaluation. Only physical exam, clinical labs, echocardiogram and AE reports will be required.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.5.1 Criteria for Removal from Protocol Therapy

3.5.1.1 Completion of protocol therapy (receipt of 5 doses of autologous AdHER2 DC vaccine).

3.5.1.2 Disease progression per **6.2**, **Response Criteria**. Note Exception: Clinically stable patients with PD at Weeks 8, 16 or 24 re-staging will be allowed to remain on protocol therapy per PI's discretion.

3.5.1.3 Patient experiences a dose limiting toxicity as outlined in Section **3.1.1**.

3.5.1.4 Patient experiences a Grade 3 or greater adverse event/toxicity *not* described in Section **3.1.1** that is deemed by the Principal Investigator as possibly, probably or definitely related to AdHER2 DC vaccination.

3.5.1.5 Patient experiences unresolved treatment-related toxicity.

3.5.1.6 Diagnosis of a secondary malignancy other than superficial basal cell or squamous cell carcinoma of the skin.

3.5.1.7 The patient experiences a treatment delay of more than 8 weeks from the scheduled target date for receipt of AdHER2 DC vaccine.

3.5.1.8 Inter-current illness or medical circumstance that in the opinion of the Principal Investigator unacceptably increases the risk of treatment. This applies to the conditions related to the cancer diagnosis that led to the enrollment in current study.

3.5.1.9 The patient is non-compliant with study requirements.

3.5.1.10 The patient becomes too ill to return for protocol specified study visits.

3.5.1.11 The patient becomes pregnant during the study.

3.5.1.12 The patient requests to be withdrawn and refuses further therapy.

3.5.1.13 The patient requires a prohibited concomitant medication.

3.5.1.14 Death

3.5.2 Continued Monitoring for Potential Cardiac Toxicity Once Off Protocol Therapy

3.5.2.1 Patient is removed from therapy for reasons outlined in Section **3.5.1**.

3.5.2.2 Patient will continue to undergo designated cardiac monitoring studies at subsequent study weeks as outlined in **Figure 11**.

3.5.2.3 Cardiac troponin levels (Weeks 0, 4, 8, 12, 16, 20, 24, 28, 32, 40 and 48) and echocardiograms (Weeks 4, 12, 20, 28, 32, 40, 48, 60. 76, 100 and 124) including HPE and ECOG assessments.

3.5.2.4 Cardiac monitoring will be performed either *at the NIH or through the patient's LMD*. For patients who come off-treatment for disease progression or other reasons, every effort will be made to capture ongoing documentation on cardiac function as is reasonably practicable, to allow long-term assessment of the impact of AdHER2 vaccination on cardiac function.

3.5.3 Off Study Criteria

3.5.3.1 Completion of scheduled study visits.

3.5.3.2 PI decision to end the study.

3.5.3.3 Patient requests to be withdrawn and refuses further study participation.

3.5.3.4 The patient is non-compliant with study requirements.

3.5.3.5 The patient becomes too ill to return for protocol specified study visits.

3.5.3.6 Documentation of rapid disease progression or clinical deterioration between the screening (including outside information) and baseline evaluations. These patients will be removed from study and precluded from receiving AdHER2 DC vaccination.

3.5.3.7 If a patient begins to take a known cardiotoxic agent the patient will come off study and we will not use data after that point.

3.5.3.8 Death

3.5.4 Off Protocol Therapy and Off Study Procedure

All subjects must be taken off study through the NCI Central Registration Office CRO). Authorized staff must notify CRO when a subject is being taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

- Patients are allowed to be on concomitant therapy with tamoxifen or aromatase inhibitors for ER+ tumor status.
- Patients may be on concomitant drugs to prevent skeletal related events (SRE), including bisphosphonates and denosumab.
- Study subjects will be allowed to take multivitamins, analgesics (NSAIDS or acetaminophen), antipyretics, and antihistamines for symptomatic relief of local or systemic injection site reactions.
- Patients are allowed to continue on medications as clinically indicated for treatment of chronic medical conditions e.g. hypertension, diabetes, hypercholesterolemia, etc.
- *Excluded* Therapy:
  - Trastuzumab, pertuzumab, lapatinib, ado-trastuzumab emtansine (TDM1) or other HER2-directed therapies *within 1 week* of study enrollment.
  - Chemotherapy: Concomitant use of chemotherapy is not allowed during this trial.
  - Anti-Cancer Radionuclides: Concomitant use of anti-cancer radionuclides is not allowed during this trial. Patients will be allowed to co-enroll in studies of novel radionuclide imaging agents e.g. ^{111}Indium CHX-A DTPA trastuzumab.
  - Secondary Hormonal Therapies: Concomitant use of supplementary hormonal treatments other than tamoxifen or aromatase inhibitors is not allowed.
  - Corticosteroids: Concomitant, *chronic systemic corticosteroids are not allowed* during this trial (excepting emergent use for clinical indications). However, the use

of inhaled corticosteroids, intranasal sprays and topical creams on limited body areas *is allowed*.

Vitamin D3 (Cholecalciferol) Supplementation

Vitamin D when ingested is metabolized in the liver to 25-OH vitamin D. Inside cells, it is metabolized further by 1-hydroxylase where it is transformed into a seco-steroid hormone that is important to a host of critical cellular and immune functions within the body. Within cells is a second enzyme 24-hydroxylase whose function is to decrease vitamin D, thereby maintaining intracellular vitamin D homeostasis. The classic function of vitamin D is to regulate calcium homeostasis and in turn, bone formation and resorption. However, additional functions of vitamin D have been demonstrated and include effects on immune response by promoting cellular apoptosis and differentiation. The exact role of vitamin D deficiency in prostate, breast, colon and other cancers has been controversial, with some laboratory studies suggesting there is a role and other epidemiological studies suggesting that there is no role or even possibly that supplementation should be avoided. In a recent study by Marshall and colleagues[34] vitamin D supplementation of 4.000 IU per day for one year was examined in men with low risk prostate cancer (Gleason score of 6, 1-6 cores positive out of 12 possible and a PSA <10) under active surveillance. After one year upon re-biopsy, 60% showed a decrease in the number of positive cores, Gleason score or both and in 6% these factors remained unchanged. In addition, PSA levels did not rise. In another study reported by Vieth and colleagues at AACR in April 2012[35], 66 men scheduled to undergo radical prostatectomy were randomly assigned to receive a daily vitamin D dose of 400, 10,000 or 40,000 IU daily for 3 to 8 weeks prior to surgery. Calcitriol levels in the prostate increased progressively with increasing vitamin D dosing and corresponded with lower levels of Ki67 as well as higher levels of specific growth-inhibitor microRNAs.

Several studies have shown that women with low vitamin D levels have an increased risk of breast cancer incidence and mortality, but research is lacking investigating vitamin D levels and prognostic variables e.g. hormone receptor status, Oncotype DX etc. in this patient population. In a case control study of 194 women s/p breast cancer surgery and 194 cancer-free controls conducted by Peppone and colleagues at the University of Rochester[36], women with breast cancer were found to have significantly lower 25-OH vitamin levels than controls (32.7ng/mL vs. 37.4 ng/mL respectively, P=0.02). Importantly, women with suboptimal 25-OH vitamin D levels (<32 ng/mL) had significantly increased odds of having ER-negative (OR = 2.59, 95% confidence interval [95% CI] = 1.08-6.23) and triple-negative cancer (OR = 3.15, 95% CI = 1.05-9.49) than those with optimal 25-OH D concentrations. In addition, women with basal-like phenotype had lower 25-OH vitamin D levels than women luminal A phenotype (24.2 ng/mL vs. 32.8 ng/mL, respectively P = 0.04). In summary, women with a more aggressive breast cancer molecular phenotype (basal-like) and worse prognostic indicators (ER- and triple-negative) had lower mean 25-OH vitamin D levels.

Given its critical role in immune function and possible role in cancer pathophysiology, all patients will have 25-OH vitamin D levels obtained at baseline. Although there is debate about the target level of 25-OH vitamin D for optimum health, most vitamin D experts agree that it should be greater than 40 ng/mL.

- *All patients with 25-OH vitamin D levels < 40 ng/mL will be initiated on oral supplements of Vitamin D3 (cholecalciferol) per standard clinical care guidelines.* 25-OH vitamin D levels will continue to be monitored at study Weeks 8, 16, 28 and 60.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

There are no pharmacokinetic studies that will be performed as part of this clinical investigation.

Evaluation of anti-HER2 antibody and cellular HER2 responses Tetramer and IFN-γ ELISPOT Assays will be performed as outlined in **Appendix 6**. If the patient does not need a confirmatory scan at Week 52, the same set of evaluations will be done at Week 60 instead of Week 52.

Characterization of vaccine-induced antibody profiles (anti-HER2 Antibody Profile) will be performed as outlined in **Appendix 6**. No additional blood to be drawn: serum will be aliquoted from blood drawn for anti-HER2 antibody studies.

Evaluation of anti-Ad5 antibody immunity will be performed as outlined in **Appendix 7**.

Characterization of soluble HER2 and anti-HER2 antibodies will be performed on the stored samples as outlined in **Appendix 8Characterization** of Soluble HER2 and Anti-HER2 Antibodies.

Exploratory correlative studies (Week 0) measuring function-associated mRNAs in whole blood to be performed by Dr. Cindy Yamamoto and circulating tumor cells (CTCs) and immune subset analysis will be performed by Jane Trepel as outlined in **Appendix 9**.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Collection and storage of Research Samples

For research samples obtained for investigation, the Clinical Support Laboratory, Leidos Biomedical Research, Inc. processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled-access building and laboratory doors are kept locked at all times. Upon specimen receipt each sample is assigned a unique, sequential laboratory accession I.D. number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession I.D. An electronic database is used to store information related to patient samples processed by the laboratory. Vial labels do not contain any personal identifier information. Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long-term storage. These facilities are operated by American Type Culture Collection (ATCC), under subcontract to Leidos Biomedical Research, Inc. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator and/or Lead Associate Investigator, specifies who has access to the collection.

When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement

(MTA) is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process. The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that an MTA has been executed prior to requesting the laboratory to ship samples outside of the NIH.

Blood, urine and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document and the proposed research has undergone prospective IRB review and approval. If new risks are associated with the research (e.g., analysis of germ line genetic mutations.) the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB-approved protocol and patient consent. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **0**.

5.2.1 Samples collected by the Blood Processing Core of the Clinical Pharmacology Program

All samples will be bar-coded, with data entered and stored in the Patient Sample Data location. Bar coded samples are stored in bar coded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (ATCC) in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the PSDM System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this specific clinical trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. Samples will be stored for future use following completion of the study unless a subject has opted out of the future use of data and specimen.

If, at any time a patient withdraws from the study and does not wish for his/her existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the

PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

No genetic/genomic analyses will be performed as part of this clinical investigation.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

1. Each patient must meet all eligibility requirements and a completed registration must be sent to the NCI Central Registration Office (CRO).
2. The Consent Document must be signed prior to registration with the CRO.
3. Treatment will be given according to protocol (on-study and treatment notes, reports of adverse events and documentation of any deviation from the study protocol).
4. Data will be entered into a secure software system (C3D Database) produced by Oracle™ Corporation (Redwood Shores, CA). Data will be collected based on protocol-specific requirements, verified for accuracy and completeness. Any hard copy data will be kept in locked secure area in the Vaccine Branch Clinical Trials Offices (Bldg 10, Rm. 4B-54).
5. Toxicity will be assessed according to the protocol using the CTCAE v4.0 that is available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
6. Response will be assessed according to the protocol and documentation of response (e.g. CT scans, laboratory reports, dated notes on tumor assessment and clinical assessment as appropriate) using Immune Response Related Criteria as outlined in Section **6.2.**
7. Vaccine report cards associated with each AdHER2 DC vaccination will be obtained for each patient.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Week 0, through 30 days after the last study intervention. Beyond 30 days after the last study intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms

- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Laboratory and clinical symptom abnormalities less than or equal to Grade 1 will not be graded or assessed for attribution to AdHER2 DC vaccine.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

Dr. Fojo will continue to have access to the data for purposes of data analysis and publication. The necessary data will be sent via encrypted email.

6.2 RESPONSE CRITERIA

Tumor measurements for response will be conducted at Weeks 8, 16, 24, 36, 48, 76, 100, and 124 with confirmatory scans obtained not less than 4 weeks following initial documentation of objective response. Adjuvant bladder cancer patients will undergo re-staging at Weeks 8, 16, 24, 36, 48, 76, 100, and 124 with confirmatory scans with confirmatory scans obtained not less than 4 weeks following initial documentation of objective response as outlined in the protocol Study Calendar in Section **3.4** and clinical evaluations will include physical examination, neck (optional), chest, abdomen and pelvis CT imaging and bone scan when clinically indicated. Dependent on the tumor site, MRI can be performed instead of CT scan. Patients with history of CNS disease will be evaluated for the necessity of follow up brain MRI. Due to the requirement of confirmational scans for the evaluation of the response at least 4 weeks after the initial documentation of the response, subsequent scan schedules can be adjusted to allow adequate interval and to avoid unnecessary radiation exposure from fixed CT scan schedules. After off-therapy or PD documentation, restaging scans are optional.

A modified Immune-Related Response Criteria (modified irRC) based on RECIST 1.1 will be used to determine the response as outlined in Section **6.2.6 Table 2** [37]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes will be used as per RECIST 1.1 criteria.

The modified irRC will be based upon RECIST 1.1 criteria (described below) except for 2 major changes:

1) require confirmation of progression by imaging at least 4 weeks after initial imaging and

2) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.

Efficacy assessments will be made by the Investigator at the investigational site. All objective responses will have confirmatory scans performed at least 4 weeks after the response was first

documented per the modified irRC convention (e.g. initial scan at Week 8, confirmatory scan at Week 12 or after etc.). The PI will review and confirm all objective responses.

6.2.1 Response Definitions

Response and progression will be evaluated in this study using the new modified irRC[37] (Section **6.2.6**.) Note: Lesions are either measurable or non-measurable using the criteria for disease parameters outlined in Section **6.2.2** provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

6.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under as ≥10 mm with CT scan
    - Scan slice thickness >5 mm: double the slice thickness
- By MRI: ≥10 mm
- With calipers on clinical exam: ≥10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area are not to be considered measurable unless they demonstrate progression.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on

occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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.

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. For certain body parts or lesions, MRI can replace CT for response evaluation and such an MRI should be optimized for the evaluation of the type and site of the disease.

Clinical lesions: Clinically visible skin lesions will be utilized to evaluate measurable disease.

Chest x-ray: Chest x-ray will not be utilized to evaluate measurable disease.

Ultrasound: Ultrasound will not be utilized as a method of measurement.

6.2.4 Evaluation of Target Lesions

- **Complete Response (CR):** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- **Partial Response (PR):** At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.
- **Progressive Disease (PD):** At least a 20% increase in the sum of the diameters of target lesions, taking as reference 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. (Note: the appearance of one or more new lesions is also considered progressions).
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

Note

- Confirmation by two consecutive observations not less than 4 weeks apart is required for CR, PR and PD
- New lesions result in unconfirmed PD. Confirmed PD is assigned only if additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target) at next assessment; the appearance of additional new lesions when none have previously been recorded, can also confirm PD

6.2.5 Evaluation of Non-Target Lesions

- **Complete Response (CR)**: Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

- **Non-CR/Non-PD**: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- **Progressive Disease (PD)**: Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel or Principal Investigator.

Note:

- Confirmation by two consecutive observations not less than 4 weeks apart was required for CR, PR and PD
- New lesions result in unconfirmed PD. Confirmed PD is assigned only if additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target) at next assessment; the appearance of additional new lesions when none have previously been recorded, can also confirm PD.

6.2.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. For the purposes of this study, best overall response will be defined according to modified irRC. **Table 2** below is modified from Wolchok JD et al, *Clin Canc Res* 2009, Hodi FS et al, J Clin Oncol 2016 and Nishino M et al, Clin Canc Res 2013[37-39] comparing RECIST 1.1 criteria, modified irRC and iRECIST. iRECIST is similar to modified irRC used in this study except that iRECIST defined unconfirmed PD and confirmed PD separately.

Table 2 Comparison of RECIST v1.1, modified irRC and iRECIST

Category	RECIST v1.1	Modified irRC	iRECIST
Measurement of tumor burden	Unidimensional	Unidimensional	Unidimensional
Target lesions	Maximum 5	Maximum 15	Maximum 5
New lesion	Results in progressive disease at first appearance	Does not always results in progressive disease at first appearance unless the sum of tumor burden fits the criteria for progressive disease	
Complete response	Disappearance of all target and nontarget lesions Lymph nodes must regress to <10 mm short axis No new lesions, confirmation required		
Partial response	≥ 30% decrease in tumor burden compared with baseline	≥ 30% decrease in tumor burden compared with baseline† Confirmation required	≥ 30% decrease in tumor burden compared with baseline
Progressive disease	≥ 20% + 5-mm absolute increase in tumor burden compared with nadir. Appearance of new lesions or progression of nontarget lesions	≥ 20% increase in tumor burden compared with baseline, with nadir, or reset baseline. New lesions added to the tumor burden Confirmation required	≥ 20% + 5-mm absolute increase in tumor burden compared with nadir. Appearance of new lesions or progression of nontarget lesions. Confirmation required
Stable disease	Neither partial response nor progressive disease		

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	

PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

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

Overall responses derived from changes in index, non-index and new lesions as demonstrated in the following table:

Measurable response	**Non-measurable response**		**Overall response using modified irRC**
Index and new, measurable lesions (tumor burden)* %	**Non-index lesions**	**New, non-measurable lesions**	
Decrease 100	Absent	Absent	CR**
Decrease 100	Stable	Any	PR**
Decrease 100	Unequivocal progression	Any	PR**
Decrease ≥ 30%	Absent / Stable	Any	PR**
Decrease ≥ 30%	Unequivocal progression	Any	PR**
Decrease < 30 to increase < 20	Absent / Stable	Any	SD
Decrease < 30 to increase < 20	Unequivocal progression	Any	SD
Increase ≥ 20	Any	Any	PD**

* Decreases assessed relative to baseline

** Assuming response (CR and PR) and progression (PD) are confirmed by a second, consecutive assessment at least 4 weeks apart.

6.2.7 Confirmatory Measurement/Duration of Response

Confirmation: To be assigned a status of a response of CR or PR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met. PD must be confirmed by a second observation no less than 4 weeks later.

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that PD is objectively documented.

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented. Time to progression (TTP) will also be recorded for patients with CR, PR and SD subsequently developing recurrent or progressive disease.

Duration of stable disease: SD is measured from the start of the treatment until the PD criteria for **progression are met.**

6.2.8 Progression Free Survival and Overall Survival

Progression-free survival (PFS) will be assessed *only* in adjuvant bladder cancer patients and in metastatic bladder cancer patients that have completed first line chemotherapy that do not have measurable disease. Overall survival (OS) is not being assessed as part of this clinical investigation.

6.2.9 Response Review

Responses will be reviewed by the Principal Investigator, Lead Associate Investigator and their designees. Since the assessment of the preliminary anti-tumor activity of autologous AdHER2 DC vaccination is only a secondary objective of this phase I, two-part dose escalation study, responses will not be reviewed by an expert(s) independent of the study.

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

The Principal Investigator and Lead Associate Investigator will be responsible for overseeing the Data and Safety Monitoring Plan. As information is gathered from this trial, clinical results will be shared with the patients. Laboratory and clinical data will be gathered on a monthly basis and any new significant observation(s) found during the course of the research that may affect patient safety or a patient's willingness to participate further will be explained at the time patients are consented for the study.

Confidentiality of information concerning participants will be maintained including in all publications and presentations resulting from this study. Names of patients or material identifying participants will not be released without permission, except as such release is required by law. Records will be maintained according to current legal requirements, and are made available for review as required by the U.S. Food and Drug Administration (FDA) or other authorized users, only under the guidelines established by the Federal Privacy Act.

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/ IRB REPORTING

7.2.1 Expedited Reporting NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of AdHER2 DC vaccine as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the AdHER2 DC vaccine, but are not fatal or life-threatening, much be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);

- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the Principal Investigator or a Lead Associate Investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The Principal Investigator will review adverse event and response data and make attribution determinations on each patient to ensure safety and data accuracy. The Principal Investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore 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 related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.03.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**.

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in Section **9**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 REPORTING PREGNANCY

8.4.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (**8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.4.2 Parental exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 90 days after the last dose of AdHER2 DC vaccine.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 90 after the last dose should, if possible, be followed up and documented.

8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSOR IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 COLLABORATIVE AGREEMENTS

10.1 TECH TRANSFER AGREEMENTS

Stored samples are being sent to Midissia for testing and characterization of soluble HER2 and anti-HER2 antibodies as a part of this protocol using a Material Transfer Agreement (MTA #42793) which was executed on 09/17/2017. There is also a CDA between Midissia and the NCI (CDA #13887-18, executed 11/10/2017). Additional tech transfers include:

- CRADA # 03048, executed 03/02/2016
- MTA #44020-28, executed 01/24/2018
- MTA #3394-13, executed 11/13/2014
- CRADA #03225, currently being negotiated

11 STATISTICAL CONSIDERATIONS

The primary objectives of this open label, single site, non-randomized, two-part, phase I study of AdHER2 DC vaccination are to determine if the fraction of patients with cardiac toxicity (if it occurs) is sufficiently low to warrant further exploration in subsequent trials and to determine an approximate estimate of the vaccine immunogenicity, as represented by the fraction of patients who develop at least a 3-fold increase in anti-HER2/neu antibody concentration (measured as mcg/mL) or a 4-fold increase in antibody dilution titers over baseline at the time points measured. An important secondary objective is to obtain a preliminary estimate of the proportion of patients who may experience tumor shrinkage or stabilization that is sufficient by modified immune response related criteria (modified irRC) to be considered stable disease, a partial response or better.

It has been reported that up to 7% of patients with HER2/*neu* expressing tumors treated with trastuzumab monotherapy for metastatic breast cancer developed clinically significant cardiac dysfunction including congestive heart failure. It would be considered acceptable if the therapeutic AdHER2 DC vaccine proposed for this study would produce no greater than that level of cardiac toxicity, but not acceptable if it were to be exceeded. In order to evaluate this using a limited number of patients, a two-part design, but incorporating an early stopping rule, will be implemented.

Statistical considerations pertaining to each part of the study are as follows:

11.1 PART I (MAXIMUM OF 30 EVALUABLE PATIENTS)

The goal of Part I is to investigate vaccine dose escalation and determine whether there is a significant, adverse safety signal regarding cardiac toxicity in patients with recurrent or progressive, metastatic solid tumors as well as adjuvant bladder cancer patients with HER2 expressing tumors that are *naive to trastuzumab, lapatinib, ado-transtuzumab and other HER2-directed therapies*, in addition to preliminary assessment of the vaccine's immunogenicity and clinical activity. Allowable tumors include breast, ovarian, colon, non-small cell lung, renal cell, bladder and prostate cancer with HER2 characterization by IHC and FISH as outlined in Section **2.1.1.14**.

Inclusion of a broad spectrum of tumors that express HER2 will facilitate more rapid accrual and allow prompt determination of the vaccine's safety and immunogenicity. However, the inclusion

of subjects with multiple tumor types that have varying levels of HER2 expression may preclude identification of an optimal biologically active dose.

During this part, vaccine safety, immunogenicity and preliminary activity will be determined for each dose level given escalating doses of vaccine as follows:

Dose Level 1 (N = 6 patients): 5×10^6 viable cells/vaccine

Dose Level 2 (N = 6 patients): 10×10^6 viable cells/vaccine

Dose Level 3 (N = 6 patients): 20×10^6 viable cells/vaccine

Expansion Cohort (N= 12 patients): 40×10^6 viable cells/vaccine

Dose escalation will proceed as long as 0 of 6 or 1 of 6 patients within a dose level have no evidence of toxicity and at least 3 of the 6 patients within a level have reached Week 12 on study. If 2 of 6 patients on any given dose level develop cardiac toxicity, that dose cohort will be closed to further accrual, no higher doses will be explored, and dose expansion of an additional 12 patients will be enrolled in *the next lower dose level* to further assess for cardiac toxicity. *The vaccine dose at which 0 to 1 of 18 treated patients in Dose Levels 1 to 3 develop cardiac toxicity will be defined as the maximum tolerated dose (MTD). If two or more patients out of 18 at a given dose have developed cardiac toxicity, then the MTD will have been exceeded at that dose, and the dose level below that one will be considered the MTD.* Since enrollment in Dose Level 3 has been reached and 0 of 6 patients has toxicity, then an expansion cohort of an additional 12 patients will be added, for a maximum targeted accrual of 30 patients in Part 1. The Expansion Cohort will be treated at 40×10^6 *viable* cells/vaccine and will be monitored for toxicity in an identical manner as previously identified. The 12 patients will be accrued at the 40x10^6 level without any planned interim stop, as cardiac toxicity is not presently anticipated given the results as of 12/11/15. With all 12 enrolled as a single cohort, there is a 4.9% probability of having 2 or more of 12 patients with cardiac toxicity if the true probability for a given patient is 3%, and 55.7% of having 2 or more of 12 with cardiac toxicity if the true probability for a given patient is 15%. Therefore, observing 0-1 of 12 with toxicity would be acceptable, and 40 x10^6 would be considered the maximum evaluated and tolerated dose. If we observe 2 or more with cardiac toxicity at or before accruing the 12^{th} patient, this exceeds a safe level. Further patients would be added subsequently, by amendment, to the next lower dose, to confirm its safety.

To ensure that the trial does not continue to accrue patients in the event that a much greater incidence of toxicity than expected would be recognized early, the following early stopping rules for accrual will be invoked in each part of the study if:

- ***Two or more of 6 patients in a given dose develop evidence of cardiac toxicity, accrual will be stopped to that dose level. Accrual expansion of an additional 12 patients will occur in the next lower dose level to further evaluate for cardiac toxicity.***
- ***Any death (unrelated to underlying primary disease) that occurs and is attributed as being possible, probably or definitely related to the investigational AdHER2 DC vaccine (FDA requirement).***

11.2 PART II (MAXIMUM OF 30 EVALUABLE PATIENTS)

Since the risk for cardiac toxicity may differ in individuals who have previously received HER2-directed therapy, the FDA is requesting that repeat dose escalation of AdHER2 DC vaccination be conducted in Part II of the study *in a population with **prior exposure** to trastuzumab, pertuzumab, lapatinib, ado-trastuzumab emtansine (TDM1) and other HER2-directed therapies.* This population will include adults with recurrent or progressive, metastatic *breast*, gastric/gastroesophageal or other cancers with 1+ to 3+ HER2/neu expression by IHC or an equivocal or positive FISH result. Vaccine dose escalation will be repeated to again determine whether there is an adverse safety signal regarding cardiac toxicity, in addition to assessment of the vaccine's immunogenicity and clinical activity. Vaccine dose escalation, administration, re-staging assessment and early stopping rules that will be invoked that are identical to that conducted in Part I.

The goal is to accrue up to 30 evaluable patients in Part II of the study and treat them with the experimental AdHER2 vaccine.

Unless an early stopping rule is invoked in either Part I or Part II of the study, the number of patients who have experienced a clinically significant cardiac toxicity will be determined to estimate the frequency of cardiac toxicity. In addition, the dose level(s) at which toxicity is observed will also be examined.

*If in part I, dose escalations have taken place in the first three dose levels and 0 of 20 patients have experienced cardiac toxicity, Part II of the study in patients with metastatic breast cancer and prior exposure to HER2-directed therapies will proceed with vaccine dosing based on the safety confirmed in Part I and as outlined in Section **3.1.2**.* A maximum accrual of 30 patients will be enrolled in Part II and patients will continue to enroll on that dose unless 3 patients have experienced cardiac toxicity.

11.3 OVERVIEW

In addition to reporting the observed fraction with toxicity, the 80% and 95% confidence intervals about this fraction will be reported.

At the conclusion of the study, the overall fractions with cardiac toxicity among 18, 24 or 30 patients enrolled at one dose or within the entire cohort will be determined. For illustrative purposes only, under a simplifying assumption that the 18-30 patients were enrolled in a single cohort without any intermediate evaluations, the following probabilities suggest that 2+/18, 2+/24 or 3+/18 with cardiac toxicity are more likely to be associated with 15% toxicity (arbitrarily chosen to substantially exceed 7%) than with 3% (arbitrarily chosen to be well below 7%).

In any given 18 patients assessed for toxicity, if two or more of the 18 develop toxicity:

- There is a 10% probability of this occurring if the true toxicity is 3%.
- There is a 78% probability of this occurring if the true toxicity is 15% (potentially unacceptable).

In any given 24 patients assessed for toxicity (6 at one dose level and 18 at a second level), if two or more of the 24 develop toxicity:

- There is a 16% probability of this occurring if the true toxicity is 3%.

- There is an 89% probability of this occurring if the true toxicity is 15% (potentially unacceptable).

If there are 30 patients assessed for toxicity (18 at one dose level plus 6 at one level and 6 at another level), if three or more of the 30 develop toxicity:

- There is a 6% probability of this occurring if the true toxicity is 3%.
- There is an 85% probability of this occurring if the true toxicity is 15% (potentially unacceptable).

As of 12/11/15, 0 of 20 patients enrolled to date that have received a minimum of 2 doses of AdHER2 DC vaccine have developed cardiac toxicity. Since we have observed 0 of 20 subjects with cardiac toxicity, this would have a 68% chance of occurring if the true probability of cardiac toxicity for a given patients was 2%; similarly, it would be 36% if the probability of cardiac toxicity was 5%, 12.2% if the probability of cardiac toxicity was 10% and 3.8% if the probability of cardiac toxicity was 15%. We are assuming that the probability of cardiac toxicity is no more than 10% since higher probabilities of toxicity are associated with a much lower chance of seeing 0 of 20 patients with cardiac toxicity as has been observed.

Since 0 of 20 patients already have been documented to have NO cardiac toxicity, and 0 to 2 patients out of 30 would have been acceptable per the original study design, a statistical estimate was performed to determine what the probability of having 0 to 2 of the next 10 patients in the Part 1 Expansion Cohort having cardiac toxicity would be. If the probability of cardiac toxicity were 2%, there would be 99.91% probability of 0 to 2 of 10 with toxicity; if it were 5%, there would be 98.8% probability; and if it were 10%, there would be 93% probability of 0 to 2 of 10 with toxicity. Thus, assuming the next 10 patients would have the same underlying per-patient probability of developing cardiac toxicity as the first 20 had, and it is likely to truly be at or below 10%, there is between a 93% and 99.9% probability that the next 10 patients enrolled in the Part I Expansion Cohort would have 0 – 2 toxicities and therefore 0 – 2 of 30 would have cardiac toxicity as per the original study design. Consequently, FDA approval was received on 12/14/15 to initiate enrollment in Part II of the study in parallel, while enrollment of the Part I Expansion Cohort is completed.

The fraction of subjects with immunogenicity, defined as a 3-fold increase in anti-HER2/neu antibody concentration (measured as mcg/mL) or a 4-fold increase in antibody dilution titers over baseline will be reported, along with 80% and 95% confidence intervals, at the following time points: Weeks 4 (after 1 vaccine dose), 8 (after 2 vaccine doses), 12 (after 3 vaccine doses), 28 (after 5 vaccine doses), 48 and 52 weeks and during extended follow-up for monitoring of cardiac adverse events at weeks 76, 100 and 124.

Finally, as a secondary objective, the fraction of subjects who haves stable disease, an objective partial response or better by modified irRC will be determined along with appropriate confidence intervals with exceptions to the PR assessment at Week 12 as noted in Sections **3.5.1**, **3.5.2** and **6.2.4**. The fraction identified in this trial will potentially be useful information for design of any subsequent trials using this vaccine in both of the patient populations being studied.

Part I of the study will accrue both males and females with HER2 expressing metastatic cancers for whom trastuzumab is not indicated and who are naïve to HER2-directed therapies. Accrual to Part II of the study will be restricted to females or males with recurrent or progressive metastatic breast, gastric/gastroesophageal or other cancers that have received clinically

indicated prior treatment with trastuzumab, pertuzumab, lapatinib, ado-trastuzumab emtansine or other HER2-directed therapies. The study will seek to accrue racially and ethnically diverse population affected by the cancer types targeted for enrollment in this study.

We expect to accrue at least 2 patients per month with expected completion of accrual within 2.5 years. If after 2 years, 20 or fewer patients have been accrued to Part I of the study, the study will be terminated for failure to accrue at a viable rate. If accrual to Part I of the study has been completed and enrollment in Part II has begun, if after 1 year, 10 or fewer patients have been accrued to Part II of the study, the study will be terminated for failure to accrue at a viable rate. To allow for the possibility of inevaluable patients, an accrual ceiling of 65 will be used.

11.4 STUDY TERMINATION

This study will be terminated for the following reasons:

- Completion of enrollment accrual and all scheduled monitoring studies for enrolled subjects.
- Development of clinically significant CTRCD toxicity (defined as a decrease in the LVEF of > 10 percentage points, to a value < 53%) in two or more of the first 10 patients enrolled in the study or four or more of the first 20 patients enrolled in the study.
- Failure to accrue study subjects in a timely manner.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects targeted for enrollment in this study have tumors that express varying levels of HER2, a well-known oncogene that when present, is characterized by more aggressive disease in certain cancers e.g. breast cancer. Significant improvements in clinical outcomes have been realized through the HER2-directed therapies such as trastuzumab and lapatinib. However, despite these successes, the usefulness of these therapies is mitigated by the requirement for a specific level of HER2 expression i.e. HER2 3+ IHC and an equivocal or positive FISH result for trastuzumab to realize clinical outcomes, the continued need for drug administration, and the ultimate development of resistance to treatment with subsequent disease progression. All of these factors point to a need for more effective therapies targeting HER2 designed to address and overcome these issues.

Subjects from both gender groups and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. Pregnant women are excluded from participation in the trial because AdHER DC vaccine may have the potential for teratogenic or abortifacient effects.

12.2 PARTICIPATION OF CHILDREN

Since this is the first in human clinical trial of this AdHER2 autologous dendritic cell vaccine and the potential safety risks are unknown, patients < 18 years of age will not be allowed to enroll in this study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The AdHER2 DC vaccine being given in this study targets both the extracellular (EC) and transmembrane ™ domains of the HER2 protein receptor. The goal of therapeutic vaccination with this product is to stimulate the patient's own immune system to make *polyclonal antibodies* (and also potentially killer cells) that recognize HER2. It is hoped that that these antibodies will have a similar beneficial effect in controlling disease and potentially prolonging survival.

Use of trastuzumab in women with breast cancer has been shown to be associated with a small, but significant risk (about 7%) for developing cardiac dysfunction. It is usually observed in patients who have previously received chemotherapy that is known to affect the heart. The risk of cardiac dysfunction is greater (about 27%) when trastuzumab is given in combination with anthracyline chemotherapy drugs such as doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan). Exactly how trastuzumab causes cardiac toxicity is unknown. It is also not known whether antibodies made by a person's own immune system will cause cardiac dysfunction. Because of this, cardiac function will be monitored very closely while receiving the vaccine treatment and for 24 months following the last dose of vaccine. In addition, there are strict stopping rules included in this study for any signals of cardiac toxicity that would appear to be exceeding the 7% frequency observed with trastuzumab monotherapy. *There are currently multiple clinical trials with vaccines using different platforms and approaches to target HER2 and to date there have been no reports of significant vaccine-associated cardiac toxicity.*

The only other likely risk associated with receiving this vaccine is local injection site reactions i.e. discomfort, itching, redness, swelling, or soreness/pain at the site where the vaccine is injected into the skin. In addition, due to the proposed mechanism of action that includes a possible acute inflammatory response at focal sites of tumor, subjects may experience discomfort at sites of metastatic disease and/or acute reactive pleural or peritoneal effusions There is a more remote potential for systemic symptoms such as fatigue, malaise or fever associated with vaccination but these are much less likely because patients are being given their own cells that have been transduced to express HER2. The AdHER2 vector used to transduce the patient's

autologous dendritic cells is not replication competent and does not integrate into the human genome. The construct has specifically omitted the oncogenic intracellular domain of HER2 to ensure additional safety.

Unlike trastuzumab or pertuzumab that are monoclonal antibodies, we anticipate that therapeutic vaccination with the AdHER2 DC vaccine will induce a *polyclonal antibody response* that targets multiple epitopes of HER2, since the immunogen contains both the extracellular (EC) and transmembrane (TM) components of HER2. This could potentially be beneficial in patients who have failed trastuzumab, lapatinib or other HER2-directed therapies due to resistance since these agents only recognize one small component of the outer portion of HER2 and as a result, many tumors become resistant. Because we anticipate the development of polyclonal antibodies that recognize multiple sites in HER2, the vaccine may also be beneficial in patients with HER2+ solid tumors as well as adjuvant bladder cancer patients at high risk of disease recurrence for whom trastuzumab is not clinically approved or for those breast cancer who don't have sufficient expression of HER2 i.e. IHC <3+ or an equivocal or positive FISH result and hence weren't eligible for trastuzumab treatment, thereby addressing an unmet medical need.

Both of the currently available HER2 therapeutics, trastuzumab and lapatinib target the extracellular component of HER2. Trastuzumab's biologic activity is believed to work by binding to HER2 receptors and interfering with signaling controlling tumor growth and division, as well as signaling the immune system to destroy the cancer cells. Lapatinib is an orally administered tyrosine kinase inhibitor of both the HER2/*neu* protein and the epidermal growth factor receptor. A third agent currently being investigated in clinical trials is pertuzumab, which is also a humanized monoclonal antibody. It has a unique function in that it prevents HER2 receptor dimerization and subsequently blocks cell signaling. All of these agents must be administered exogenously and continuously for beneficial effect at great cost and often with significant discomfort to the patient.

All care will be taken to minimize side effects. Patients will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of patients will be recorded in the patient chart. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the NIH Clinical Center, Bethesda, Maryland.

Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. In all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) or other regulatory authorities may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration to register and verify patients' eligibility.

12.5 RISKS/BENEFITS ANALYSIS

Subjects (including subjects unable to give consent) undergoing therapeutic autologous AdHER2 DC vaccination are likely to experience local injection site reactions commonly associated with the delivery of all vaccines i.e. discomfort, itching, redness, swelling, and/or soreness/pain at the injection site. The potential for adverse systemic symptoms such as fatigue, malaise, fever or acute allergic reactions is unknown but is less likely because patients are being given their own cells. However, because patients are being given their own cells as well as a "self" tumor

antigen (HER2), there is the potential for inducing antibodies that react with the patient's own tissues resulting in autoimmune disease. This has not been described with other HER2 vaccines that have been studied but patients will be monitored for the development of autoimmune disease.

The greatest concern associated with this therapeutic vaccine platform is the potential development of vaccine-induced antibodies that result in cardiac dysfunction and toxicity. Unlike trastuzumab, where therapy can be withdrawn, there is no way to "turn off" the immune system without significant adverse consequences. Because of this, cardiac function will be monitored very closely while receiving the vaccine treatment and for 24 months following the last dose of vaccine. In addition, stringent stopping rules are in place in the event of any early (or even late) signals of cardiac toxicity.

The patients eligible to enroll in Part I of this study have recurrent or progressive metastatic disease as well as adjuvant bladder cancer patients with HER2 expressing tumors or naïve to HER2 targeted therapies that are not candidates for currently available HER2-directed therapies such as trastuzumab and lapatinib. Thus, therapeutic vaccination with the AdHER2 DC vaccine platform proposed in thus study may result in the development of polyclonal antibodies that successfully target and interfere with HER2 expressed at lower levels and on tumors other than breast cancer and provide a potential direct benefit.

The patients eligible to enroll in Part II of this study have recurrent or progressive metastatic breast cancer, gastric or gastroesophageal junction cancer have high HER2 expression and have failed or progressed on prior FDA-approved HER2-directed therapies such as trastuzumab, pertuzumab, lapatinib, ado-trastuzumab emtansine (TDM1) or other HER2- directed agents (e.g. MGAH22). The induction of polyclonal antibodies associated with the AdHER2 DC vaccine platform may provide recognition of new HER2 targets and overcome resistance to trastuzumab and lapatinib with the potential for beneficial clinical outcomes.

In addition, the results may help the investigators learn more about the disease and develop new treatments for patients with this disease.

Overall, preliminary findings support a favorable benefit:risk ratio for the use of AdHER DC vaccine in these patients with tumors with 1-3+ HER2/neu expression. Several measures, including project-specific safety related inclusion/exclusion criteria, regular physical examinations, laboratory testing, etc., throughout the study and toxicity management guidelines have been incorporated into the study protocol to mitigate any potential or identified risks.

NOTE: This same risks/benefits analysis applies to all study populations included in this clinical study.

12.6 CONSENT PROCESS AND DOCUMENTATION

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, and the potential benefits will be carefully explained to the patient and the patient's advocate. It will be stated clearly that participation in the research study is voluntary and that participants can withdraw from the study without losing benefits they would otherwise be entitled to. The patient and family members will be encouraged to ask questions. This process will include a general description of the disease process, as well as a description of the patient's expected clinical course. Enrollment on this study will only occur if the patient meets all eligibility criteria.

Eligible patients will be presented with a detailed description of the study protocol plan and treatment and provided with a copy of the IRB-approved Informed Consent to review in advance of any discussions with the clinical trials team. The specific requirements, research objectives, risks, alternatives, time commitments and potential benefits will be reviewed with the patient. The patient will be reassured that participation in this study is entirely voluntary and that they may withdraw or decide against receipt of vaccination at any time without adverse consequences. All questions about the study will be answered and alternatives to participation will also be discussed. The Principal Investigator, Lead Associate Investigator or their designee is responsible for obtaining a signed protocol Informed Consent using the current version approved by the NIH Intramural IRB and posted on the web. The original signed informed consent will be placed in the patient's medical record and a copy will be provided to the patient.

12.6.1 Telephone consent procedure

Consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented in the medical record.

13 PHARMACEUTICAL INFORMATION

13.1 AD5F35HER2ECTM (ADHER2)

13.1.1 Source

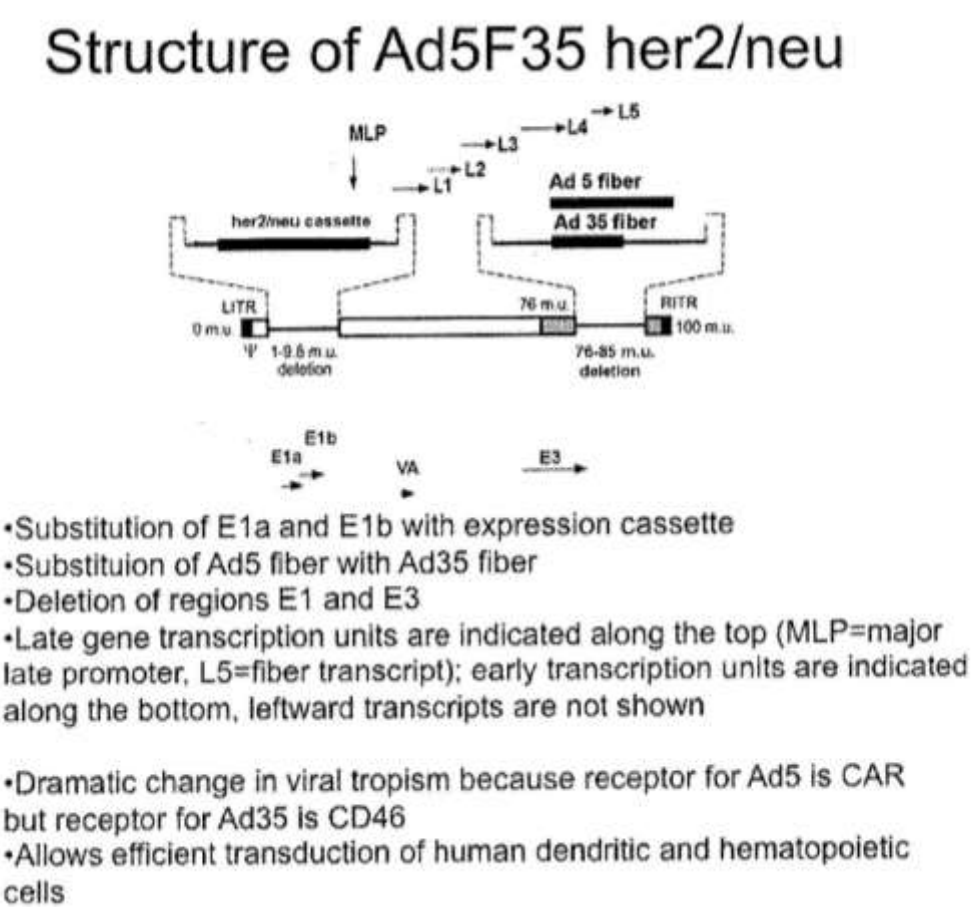
13.1.1.1 Product/Vector Description

Product Name: Ad5f35HER2ECTM

Vector Derivation

The Ad5f35HER2ECTM vector was produced under cGMP conditions in the Vector Production Facility (VPF) of the Center for Cell and Gene Therapy (CAGT) at Baylor College of Medicine. The knob and fiber of Ad35 is substituted yielding a recombinant Ad5f35 vector that allows for greater viral tropism and more efficient transduction of human dendritic cells (DC) that are the proposed platform for this vaccine as outlined in **Figure 12.**

Figure 12: Ad5f35 Human HER2/*neu* ECTM Vector Product



13.1.1.2 Vector Manufacturing Summary

The vector was manufactured under cGMP conditions by the Baylor College of Medicine Center for Cell and Gene Therapy (CAGT). The adenoviral vector was initially purified by two rounds of plaque isolation and then serially propagated on human embryonic kidney cells (HEK-293). The final amplified adenoviral vector was purified by ultracentrifugation using double cesium chloride gradients. Residual cesium chloride was removed by dialysis of the solution containing the vector against the formulation buffer. Refer to adenoviral vector cGMP production as outlined in **Appendix 10.**

An initial Master Virus Bank of Ad5f35HER2ECTM was generated (VM1003) and underwent two rounds of plaque purification with ultimate generation of purified Master Virus Bank Bulk Vector (VMB1003) which was subsequently used to produce the first clinical clot of vector that was then vialed for clinical use. The Ad5f35HER2ECTM clinical product is labeled VMC1003 and contains 1x10e12 viral particles/mL and 0.2mL/vial. A map of the Ad5f35HER2ECTM vector is outlined in **Appendix 11**.

13.1.1.3 Vector Quality Assurance/Quality Control (QA/QC)

Per contractual requirements with the Baylor College of Medicine CAGT, the manufacture and testing of the final clinical Ad5f35HER2ECTM vector product (both bulk and vialed) was conducted under current Good Manufacturing Practice (cGMP) guidelines in accordance with

the cGMP Regulations outlined in 21 CFR Parts 210 and 211. CAGT is required to prepare and complete documentation as manufacturing tasks have been performed and this documentation is subsequently reviewed by the CAGT QA division. This documentation includes sequence verification, the manufacturing batch records, certificate of analysis and any other manufacturing related information for CMC documentation required for IND filing.

13.1.2 Toxicity

There is no known toxicity of the Ad5f35HER2ECTM vector. It is a replication incompetent vector that does not integrate into the genomic DNA of the host cells it is used to transduce/transfect. Transduction of dendritic cells is associated with some reduction in cell viability that his highly donor dependent based on pre-clinical studies characterizing the proposed transduction process that will be used in this study in normal donors. The Ad5f35HER2ECTM vector has undergone replication capacity assay (RCA) assay testing that has been reviewed by the FDA and determined to be acceptable for its proposed use in this study.

13.1.3 Formulation and Preparation

13.1.3.1 Vector Vialing and Storage

The product labeling for **the *first* clinical lot of Ad5f35HER2ECTM vector (VMC1003)** that was vialed on 12/21/10 and **that will be used in the current study** for transduction of autologous dendritic cells to generate vaccines for this study is:

Ad5f35-Her2-ECTM
Lot: VMC1003
Conc: 1E+12 vp/mL Vol: 0.2 mL
Store: < = -70C Exp: 12/21/2015 (stability studies pending)
Made by CAGT Baylor, Houston, Texas
New Drug For Investigational Use Only
Total: 519 vials

There is also a Virus Master Archive for this clinical lot (VMA1003): 1E=12 vp/ml, 1.0mL/vial. Total: 30 vials.

A second clinical lot of vector (VEC1003.1.11) was produced and vialed on 02/09/12. It will be *available for use in future investigational studies* of the Ad5f35HER2ECTM vector as well as in this study should the first lot be used up or become unusable. This second clinical lot is labeled as follows:

Ad5f35-Her2-ECTM
Lot: VEC1003.1.11
Conc: 1E+12 vp/mL Volume: 0.2 mL
Store: < = -70C Date Fill: 02/09/2012
Made by CAGT Baylor, Houston, Texas
New Drug For Investigational Use Only

The vialed vector is stored below -70C at the NIH Clinical Center Investigational Drug Management Research Section (IDMRS). Investigational drug supply of vector used for manufacture of autologous HER2 DC vaccines will be tracked and documented as per regulatory requirements.

13.1.3.2 **Proposed Vector Product Use and Formulation:** Vector-Transduced Autologous Dendritic Cell Vaccine

13.1.3.3 **Product Name:** Autologous Ad5f35HER2ECTM transduced dendritic cell vaccine (AdHER2 DC Vaccine)

13.1.3.4 Dendritic Cell Vaccine Manufacturing Summary

The autologous AdHER2 DC vaccine will be manufactured by the Department of Transfusion Medicine (DTM) at the NIH Clinical Center in Bethesda, MD:

National Institutes of Health
NIH Clinical Center
Department of Transfusion Medicine
10 Center Drive-MSC 1184
Building 10, Room 1C711
Bethesda, MD 20892-1184

The Sponsor-Investigator held IND that will be submitted for this product will include a Letter of Authorization to Cross-Reference BB-MF 11054 from the Master File Type V for Facility, Operational and Quality Systems for the Manufacture of Cellular Therapy Products that is held by DTM.

Refer to Appendix 3 for the DTM Standard Operating Procedure for Manufacture of the AdHER2DC vaccine

13.1.4 Stability and Storage

Vector Stability and Release Testing

v. 10/16/09 FINAL

TABLE 1: VIRAL VECTOR STABILITY STUDY

Test	Time Points & Maximum Number of Vials to be used						
	0 months	6 months REMOVED	12 months	24 months	36 months	48 months	60 months
Particle count by OD	1	1	1	1	1	1	1
Infectious titer by CPE	2	2	2	2	2	2	2
Sterility (container integrity)	3	3	3	3	3	3	3
Functionality	2	2	2	2	2	2	2
Total	8	8	8	8	8	8	8

Reference source: Protocol to Monitor the Stability of the Adenovirus Reference Material [ARM] (www.wilbio.com)

Particle count data is valid for all averaged readings +/- 20% of the total particles aliquoted.

Infectious titer data is valid for all averaged replicates

T=0 is the date of storage after aliquoting

Initiate assays +/- 2 weeks of time point for points < 24 months.

Initiate assays +/- 4 weeks of time point for points ≥ 24 months.

Vector stability and release testing is being conducted at 0 months, 12 months and yearly thereafter as outlined below.

Additional testing is conducted for infectious units (IU), viral particles (VP). USP sterility, endotoxin, pH, transgene expression and restriction digest.

Vialed vector is stored at < -70 degrees Celsius.

13.1.5 Administration Procedures

Refer to **Appendix 3** for details regarding Ad5f35HER2ECTM vector use in the transduction of autologous dendritic cells and generation of final vaccine product.

13.1.6 Incompatibilities

The Ad5f35HER2ECTM vector has no known incompatibilities in culture.

13.2 SARGRAMOSTIM

13.2.1 Source:

The GM-CSF to be used in this study is glycosylated, recombinant human Granulocyte-Macrophage Colony Stimulating Factor (rhu GM-CSF) (brand name Leukine®). This rhu GM-CSF is an altered form of the native molecule: the position 23 arginine has been replaced with a leucine to facilitate expression of the protein in yeast (Saccharomyces cerevisiae) and the carbohydrate moiety may be different from the native protein. The Clinical Center Pharmacy will purchase commercially available sargramostim. Rhu GM-CSF will be used both as an ancillary product for the maturation of dendritic cells and for administration to patients with peptide vaccination.

13.2.2 Formulation and Preparation:

Lyophilized LEUKINE is a sterile, white, preservative-free powder (250 mcg) that requires reconstitution with 1 mL Sterile Water for Injection, USP or 1 mL Bacteriostatic Water for Injection, USP. Reconstituted lyophilized LEUKINE is a clear, colorless liquid suitable for subcutaneous injection or intravenous infusion. The vial of lyophilized LEUKINE contains 250 mcg (1.4×10^6 IU/vial) sargramostim, 40 mg/mL mannitol, USP; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, USP, as excipients. Biological potency is expressed in International Units (IU) as tested against the WHO First International Reference Standard. The specific activity of LEUKINE is approximately 5.6×10^6 IU/mg.

13.2.3 Stability and Storage:

Lyophilized LEUKINE should be refrigerated at 2 to 8° C (36 to 46° F) and is stable for at least eighteen months. It should not be frozen, shaken or used beyond the expiration date printed on the vial. LEUKINE reconstituted with 1.0 mL of Sterile Water for Injection, USP (without preservative) should be administered as soon as possible and within 6 hours following reconstitution. Reconstituted solutions prepared with Bacteriostatic Water for Injection USP (0.9% benzyl alcohol) may be stored for up to 20 days at 2 to 8° C prior to use. The contents of vials reconstituted with different diluents should not be mixed together.

13.2.4 Administration Procedures:

Lyophilized LEUKINE will be used *in vitro* by the Department of Transfusion Medicine for the generation of dendritic cells by the Department of Transfusion Medicine.

13.2.5 Incompatibilities:

Refer to the package insert/PDR/Formulary.

13.3 INTERLEUKIN-4 CELLGENIX

13.3.1 Source:

Interleukin-4 (IL-4) used in this study is investigational. It is manufactured and supplied by CellGenix (Master File cross reference BB-MF 11269). It will be used as an ancillary product to mature dendritic cells *in vitro* and will not be administered directly to patients. IL-4 exerts important effects on B cells, T cells, macrophages, eosinophils, hematopoietic progenitor cells, endothelial cells and promotes the maturation of dendritic cells. The complementary DNA clone (cDNA), when expressed in E.coli yields a 130 amino acid residules with a molecular mass of 14 kDa. IL-4 is a highly purified (≥ 97% as determined by SDS-PAGE), sterile, water-soluble protein.

13.3.2 Formulation and Preparation:

RhIL-4 Sterile Powder for Injection is supplied in a 50mcg vial as a sterile lyophilized powder formulated with glycine, human serum albumin, citric acid, and sodium citrate. Approximately 1.2 mL of Sterile Water for Injection USP should be added to each vial of rhIL-4 Sterile Powder for Injection. The vial should be gently agitated to completely dissolve the powder and should be inspected visually for discoloration and particulates prior to use.

13.3.3 Stability and Storage:

The product both intact and reconstituted vial should be stored at -20°C to -80°C and should be used within 24 hours.

13.3.4 Administration Procedures:

To be used in dendritic cell culture, not administered directly to patients.

13.3.5 Incompatibilities:

None known in culture.

13.4 KLH (KEYHOLE LIMPET HEMOCYANIN)

13.4.1 Source

KLH protein (Product Code KLH-02NV) was purchased from Stellar Biotechnologies, Inc. and then vialed by Element SL Pharma. It is stored in Center for Cellular Engineering in Department of Transfusion Medicine, NIH Clinical Center for use in dendritic cell culture. The KLH formulation to be utilized by NIH has been purified as native molecules and designated as High Molecular Weight KLH (HMW-KLH).

The only source for KLH is the hemolymph of Giant Keyhole Limpets, Megathura crenulata, an ocean mollusk. Stellar's clinical grade HWM-KLH is purified under cGMP conditions, and sourced from giant keyhole limpets cultured by Stellar under controlled aquaculture systems.

KLH protein is expressed in two subunit isoforms (KLH1 and KLH2) of approximately 360,000 and 400,000 monomeric molecular weight, respectively. The KLH monomers are each composed of 7 or 8 functional unit domains; each functional unit contains an oxygen binding site containing two copper atoms. Both KLH isoforms assemble into native homo-decamers and didecamers of 4,000,000 to 8,000,000 molecular weight in hemolymph.

13.4.2 Formulation and Preparation:

Stellar high molecular weight KLH is provided in soluble form in a buffer solution that is composed of 10mM sodium phosphate, 135mM NaCl, 1mM $CaCl_2$ and 0.5mM $MgCl_2$ as a bulk drug substance at 5mg/mL and then diluted and vialed into single use vials at 2mg/mL, 250 µL/vial by Element SL Pharma, a vendor qualified by NIH Sterile Product for Human Administration (SPHA) committee.

13.4.3 Stability and Storage:

HMW-KLH is stable for at least 12 months when stored at 2 to 8°C. A stability testing program is in place.

13.4.4 Administration Procedures:

HMW-KLH will be used in vitro by the Department of Transfusion Medicine at a concentration of 10mcg/mL for the generation of dendritic cells. Any excess culture media will be removed Cells before administration.

13.4.5 Incompatibilities:

No information available.

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15 APPENDICES

15.1 APPENDIX 1: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. 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).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

15.2 APPENDIX 2: NEW YORK HEART ASSOCIATION(NYHA) FUNCTIONAL CLASSIFICATION

The New York Heart Association (NYHA) Functional Classification in a Patient with Heart Disease

Overview: The New York Heart Association (NYHA) developed a functional classification for patients with heart disease.

Patients: Heart disease must be present.

Parameters:

(1) Limitations on physical activity

(2) Symptoms (undue fatigue palpitations dyspnea and/or anginal pain) with ordinary physical activity

(3) Status at rest

Limitations on Physical Activity	**Symptoms with Ordinary Physical Activity**	**Status at Rest**	**Class**
None	None	Comfortable	I
Slight	Symptomatic with ordinary activities	Comfortable	II
Marked	Symptomatic at less than ordinary levels of activity	Comfortable	III
Unable to perform any activity	Discomfort with any activity	Symptomatic at rest	IV

In addition the level of objective evidence was also classified:

Objective Evidence of Cardiovascular Disease	**Class**
No evidence of disease	A
Minimal disease	B
Moderate disease	C
Severe disease	D

15.3 APPENDIX 3: STANDARD OPERATING PROCEDURE FOR MANUFACTURE OF ADHER2 AUTOLOGOUS DENDRITIC CELL VACCINE

Standard Operating Procedure and Dendritic Cell Manufacturing Summary

The autologous AdHER2 DC vaccine will be manufactured by the Department of Transfusion Medicine (DTM) at the NIH Clinical Center in Bethesda, MD:

National Institutes of Health
NIH Clinical Center
Department of Transfusion Medicine
10 Center Drive-MSC 1184
Building 10, Room 1C711
Bethesda, MD 20892-1184

The Sponsor-Investigator held IND that will be submitted for this product will include a Letter of Authorization to Cross-Reference BB-MF 11054 from the Master File Type V for Facility, Operational and Quality Systems for the Manufacture of Cellular Therapy Products that is held by DTM.

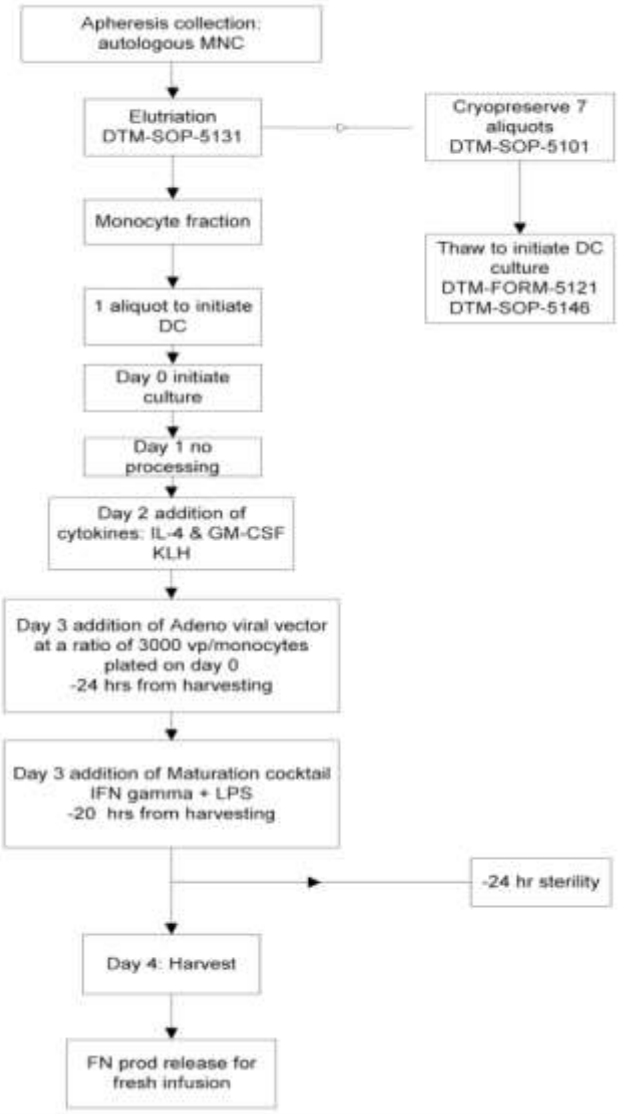
Autologous Cell Harvest

Blood collection will be by standard apheresis: up to 18L of whole blood will be processed in order to collect peripheral blood mononuclear cells (MNC) with a target yield of at least 2.2×10^9 monocytes. Lymphocytes will also be cryopreserved for immunologic assays. Apheresis will be performed in the Clinical Center (CC) Department of Transfusion Medicine (DTM) using approved standard operating procedures. Bilateral peripheral venous access or a venous dual lumen catheter will be used for apheresis. Prophylactic intravenous CaCl2 and MgSO4 infusions may be administered during apheresis to treat or prevent citrate toxicity at the discretion of the DTM physician per routine. Autologous monocytes for dendritic cell culture will be enriched from peripheral blood MNC apheresis collections by counter-flow elutriation, aliquoted into multiple vials and cryopreserved for future preparation of the dendritic cell products: target of at least 6 vials with ~ 333 x 10^6 cells/vial. Additional apheresis will be offered per investigator's discretion if the collected cells or plasma are not sufficient to manufacture at least 5 vaccine doses. All cell processing will be conducted in accordance with approved DTM policies and procedures.

AdHER2 Transduced Dendritic Cells

Autologous AdHER2 DC vaccines will be prepared under GMP conditions from cryopreserved patient monocytes as outlined in the accompanying AdHER2 DC manufacturing schema. The strength (vp/mL) of AdHER2 vector will be adjusted per most recent stability test result. The label on the vial reflects the strength at initial release. The range of ± 50% of the strength reported on the product label will be deemed acceptable to use in vaccine manufacturing with PI review of updated annual stability test result and approval.

15.4 APPENDIX 4: ADHER2 DC VACCINE MANUFACTURING SCHEMA



Description of AdHER2 DC Vaccine Manufacture

Day 0 Prior to the scheduled administration of the Dendritic Cells vaccine, one cryopreserved monocytes enriched aliquot will be thawed, washed and evaluated for cell count and viability. Monocytes will be resuspended in media containing 90% RPMI-1640, 10% auto HF plasma or allo HIF AB plasma, 2000 IU/mL IL-4 2000 IU/mL rGM-CSF and 10 mcg/mL gentamicin.

Day 1 No processing required

Day 2 Fresh media and cytokines (2000 IU/mL rIL-4, 2000 IU/mL of rGM-CSF) will be added to the culture; KLH will be added at a final concentration 10 mcg/mL.

Day 3 The AdHER2 vector will be added at a ratio of 3000 viral particles per monocytes plated on Day 0; vector viral particles will be added at -24 hrs from harvesting.

Vector transfection will occur over 4 hours before the addition of maturation cocktail at -20 hrs from harvesting. Maturation cocktail consist of LPS and IFN-γ at a final concentration of 30 ng/mL and 1000 IU/mL, respectively.

Removal of sample for -24 hours sterility testing.

Day 4 Final harvest, packaging and lot release testing of autologous AdHER2 DC vaccine preparation. The final transfected, volume-reduced mature AdHER2 DC vaccine product will be prepared in sterile syringes for fresh administration intradermally.

The AdHER2 DC vaccine product will be prepared as summarized above using a comprehensive, validated manufacturing process described in the DTM Standard Operating Procedures (SOP). Detailed SOP for processing, labeling, storage, and quality assays are also available on site in the Cell Processing Section of DTM. All DTM SOPs related to the manufacture of the AdHER2 DC vaccine will be included in the Sponsor-Investigator held IND to be submitted for the product.

Vaccine Processing Steps and In Process Testing

Multiple sample and assay timelines for the manufacturing processes of the AdHER2 DC vaccine are detailed in the CMC file in DTM and include:

- Timeline for processing and in process testing of autologous mononuclear cells to generate monocyte and lymphocyte fractions via elutriation for cryopreservation.
- Timeline for processing and in process testing during AdHER2 DC vaccine manufacture.

Vaccine Manufacture Critical Reagents

Critical reagents in the manufacture of autologous AdHER2 DC vaccine include interleukin-4 (IL-4), KLH (Keyhole Limpet Hemocyanin), sagramostim (GM-CSF) and reference endotoxin (E. coli). **Detailed information on these agents is outlined in protocol Section 13, Pharmaceutical Information.** Letters of Authorization to cross-reference the master files of these agents will be included with the formal IND submission for the product.

Vaccine Release Testing

Release testing will include not only safety testing (microbiological testing) but will also include any product characterization that is being done such as identity, purity, and potency testing. The following tests will be performed related to AdHER2 DC vaccine release criteria and quality control:

a) Vaccine Product Safety Testing

1. Sterility (bacterial and fungal) testing of all in-process and final product samples of the AdHER2 DC vaccine product will be done by the Bactec automated blood culture system. *Although final product sterility testing will be performed, results will not be available as a release criterion.*

   DTM has completed and published the results of a study demonstrating that both the BacT/Alert (BioMerieux) and the Bactec (Becton Dickinson) automated culture systems are superior to the CFR/USP method for sterility testing of cell therapy products (seeded

study; *Khuu HM et al. Cytotherapy 2004; 6(3): 183-195*). They also published a parallel testing study of CFR/USP vs. Bactec, for sterility testing of actual cell therapy products from their facility. Results of the parallel studies show that the automated culture methods had fewer false positives than CFR/USP (*Khuu HM et al. Transfusion 2006; 46: 2071-82*). Based on the analysis of both studies, DTM has sufficient data to implement the Bactec system as the replacement for the CFR/USP method.

Justification for use of automated system in place of CFR/USP method is based on the results of the studies described above. In the seeded study, 10 different organisms were seeded into mononuclear cell products suspended in 6 different media, one of which was X-Vivo20 (which contains gentamicin), and one of which was our standard freeze mix (Plasmalyte A, human serum albumin, Pentastarch, DMSO, Dornase, and heparin), which is used to cryopreserve all products in this protocol. The antibiotic level tested in the seeded study is **50mg/mL** and this level was shown to be non-inhibitory in the automated culture system. This is the same level in the samples for sterility for the product in this IND.

2. Negative gram stain must be documented
3. Sterility of -24hrs in process sample must be documented
4. Mycoplasma testing will be done by Mycoplasma PCR method (Roche) but results will not be available for use as a release criterion
5. Endotoxin testing will be done by the KQCL method; endotoxin level must be < 5EU/ml.
6. Transfusion-transmitted disease (TTD) testing will be performed using methods described in our Master File CC-MF-11054 (Attachment 8-A), with the following modification. Testing is performed by Blood Systems Laboratories in Tempe, AZ CLIA Reg #03D0911463). Agents include T. cruzi as well as those listed. Syphilis is tested using PK-TP assay, instead of RPR.

b) Vaccine Product Characterization

1. Total cell number as determined using an automatic counter.
2. Viability using Trypan blue dye exclusion with a minimum viability of ≥ 60% as release criteria.
3. Cell phenotype and expression for HER2*neu* by FACS.
   Phenotype markers will include: **CD14, CD38**, CD54, **CD83**, **CD86**, **CD 340 (HER2neu),** CCR7, HLA-DR. **Key phenotype markers in bold.**
4. Day 3 in process DC supernatants prior to transfection with adenovirus for cytokine assessments by multiplex and proteomic array (for research purposes).

Vaccine Product Release and Action Plans for Positive Results on Safety Testing

General action plans for dealing with positive tests for sterility, mycoplasma, and endotoxin testing are presented in our Master File BB-MF-11054 (Attachments 9-B through 9-D). As noted above, not all safety testing will be available prior to release of the AdHER2 DC vaccine product. For testing with final results available, results must be within pre-specified limits and documented on the Certificate of Analysis before the product is released for administration to the patient.

Vaccine Final Product Preparation Steps (fresh infusion)

The final AdHER2 DC vaccine product will be harvested from the 5-day culture product and packaged for fresh infusion on the same day.

Vaccine Product Tracking and Labeling

The system for labeling and tracking of all products in this IND will be the same as the general system used in the DTM Cell Processing Facility, described in their Master File BB-MF-11054, section XI.

Vaccine Product Administration

Autologous AdHER2 DC vaccines will be harvested from the 5-day culture product as previously described and packaged for fresh administration on the same day according to DTM Standard Operating Procedures. The protocol specified AdHER2 DC vaccine dose of 5 X 10^6 *viable* cells, 10 X 10^6 *viable* cells, 20 X 10^6 *viable* cells or 40 X 10^6 *viable* cells will be concentrated in a final total volume of 1.0mL for intradermal administration immediately upon receipt in the clinical setting. Post packaging testing of DC vaccines produced by DTM indicates that the product should be stable for at least 2 hours.

Patients will have vital signs monitored for one hour following administration of the first dose of AdHER2 DC vaccine and for 15 minutes with all subsequent doses if there are no significant acute adverse systemic clinical events (e.g. fever, hypotension, wheezing, rash/urticaria) documented after the first dose. It is anticipated that the primary adverse events associated with AdHER2 DC vaccination will be local injection site reactions, and possibly symptoms related to local anti-tumor inflammatory responses at sites of primary and metastatic disease including focal organ discomfort and/or reactive pleural and peritoneal effusions.

15.5 APPENDIX 5: ADHER2 DENTRITIC CELL VACCINE REPORT CARD

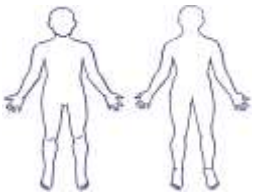
Date/Time Vaccine Administered: _____ Administered By: _____

Study ☐Part I ☐Part II Vaccine Dose ☐$10e^6$ cells ☐$20e^6$ cells ☐$40e^6$ cells

Study Week _____ Vaccine Dose # ☐1☐ 2☐ 3☐ 4 ☐5

	Injection Site: Forearm	**Injection Volume (mL)**	***ID Wheal Present?***	**DA Y 1**	**DA Y 2**	**DA Y 3**	**DA Y 4**	**DA Y 5**	**Date resolved**
Site #1	☐Left ☐Right		☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	
Site #2	☐Left ☐Right		☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	
Site #3	☐Left ☐Right		☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	
Site #4	☐Left ☐Right		☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	

Total Injection Volume: _____



Injection Site Reaction Grading (NCI CTCAE v4.03)

☐ 0 No Reaction
☐ 1 Tenderness *with or without* associated warmth, redness, itching.
☐ 2 Pain, swelling (edema), lipodystrophy (shrunken area of the skin due to loss of fat), or phlebitis (inflammation of veins in the skin).
☐ 3 Ulceration or necrosis (skin break down); severe tissue damage. Operative intervention indicated.
☐ 4 Life-threatening consequences. Urgent intervention indicated.

Note: If you have a reaction that lasts longer than 5 days please continue to describe it on the back of this page.

Print Name:_____

Signature:_____

15.6 APPENDIX 6ANTI-HER2 ANTIBODY AND CELLULAR RESPONSES

The majority of the testing on this study will be done in the NIH Clinical Center clinical laboratory following their guidelines for blood collection and tube type. The appropriate tube for uncommon laboratory tests and immunologic research specimens and where they should be sent are as follows:

Quantitative Anti-HER2 Antibody Testing: Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124

Purpose: To determine the immunogenicity of autologous Ad HER2 dendritic cell vaccination as measured by a 3-fold increase in anti-HER2/neu antibody concentration (measured as mcg/mL) or a 4-fold increase in antibody dilution titers over baseline.

Specimen Processing: 1 10mL Red Top Clot activator
Specimens will be processed by the Clinical Support Laboratory Leidos Biomedical Research, Inc. Serum will be aliquoted into vials and cryopreserved until ready for interrogation in batched specimen assays.

Characterization of Vaccine-Induced Antibody Profiles Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124

Purpose: To characterize vaccine-induced antibody profiles using HER2 peptide microarrays, examining reactivity to HER2 extracellular (EC), transmembrane (TM) and intracellular (IC) domains to document evidence of epitope spreading.

To characterize immunoglobulin sub-classes of anti-HER2 antibodies.

Specimen Processing: No additional specimens to be drawn.
Will utilize clinical specimens drawn and processed for quantitative anti-HER2 antibody testing.

Cellular Responses to HER2/*neu:*
Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124Tetramer & IFN-γ ELISPOT Assays:

3 10mL Green Top Heparinized Tubes (30 mL total)

Send via Frederick Courier to NCI Frederick Clinical Support Laboratory for specimen processing and freezing.

Tetramer Assay: Will be performed by the flow cytometry unit in the NCI Frederick Clinical Support Laboratory or Vaccine Branch Flowcytometry Core.

IFN-γ ELISPOT Assay: Will be performed in the Laboratory of Cell Mediated Immunity or Vaccine Branch Flowcytometry Core.

The Clinical Support Laboratory, Leidos Biomedical Research, Inc., processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Upon specimen receipt, each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID. An electronic database is used to store information related to patient samples processed by the laboratory. Vial labels do not contain any personal identifier information. Samples are stored inventoried in locked laboratory freezers and are

routinely transferred to the NCI-Frederick repository facilities for long-term storage. These facilities are operated by ATCC. under subcontract to Leidos Biomedical Research, Inc. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator is responsible for the collections, typically the protocol Principal Investigator who has access to the collection. Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document, the patient has provided consent for the research use of their clinical specimens, and the proposed research has undergone prospective IRB review and approval. The NIH Intramural IRB will be notified when samples are destroyed.

15.7 APPENDIX 7EVALUATION OF ANTI-AD5 ANTIBODY IMMUNITY

An intramural scientific collaborator or scientific contractor to perform these studies to be determined.

Anti-Ad5 antibody ELISA assessments will be performed at

Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124

to determine in an exploratory manner if:

1. AdHER2 DC vaccination is associated with a measurable increase in anti-Ad5 antibody over time.
2. Pre-existing levels of anti-Ad5 antibodies inversely correlate with vaccine immunogenicity as measured by anti-HER2 antibody levels.

We will store the samples in a -80°C freezer until batched shipped at which point they will be processed.

15.8 APPENDIX 8CHARACTERIZATION OF SOLUBLE HER2 AND ANTI-HER2 ANTIBODIES

Testing and characterization of soluble HER2 and anti-HER2 antibodies will be performed by a third party Midissia or other third-party collaborator using samples stored as described in **Appendix 6**. Midissia is a developing biopharma company that has licensed the AdHER2 DC platform in combination with a checkpoint inhibitor.

15.9 APPENDIX 9EXPLORATORY CORRELATIVE STUDIES 1 of 2

Measurement of Function-Associated mRNAs in Whole Blood: Hitachi Chemical

Function-Associated mRNAs: (Week -0) One 6 mL green top sodium heparin tube

For <u>Prediction</u> of Responses:

- Blood will be drawn into one 6 mL green top tube.
- Add 180 µL each of blood into one 8-well strip. Strips contain PHA, HAG, PBS, DMSO, rIL2, CEF, LPS/ZA, and HER2).
- Incubate at 37°C for 4 hours.
- Store in -80°C (+/- 10°C) freezer until ready for shipment to Hitachi Chemical
- **Note:** ***specimen should be drawn DAY OF VACCINE before 1pm*** to allow adequate time for specimen processing and four hour incubation by Trepel lab staff.

For <u>Characterization</u> of Responses will be assessed after "Prediction of Responses" described above confirms the list of mRNA to pursue.

- Blood will be drawn into one 6 mL green top tube.
- Approximately 1% ($2x10^5$ cells) of final AdHER2 DC vaccine product in 0.1mL of standard suspension (taken at the same time as DC product QC test samples) and 0.2mL of heparinized whole blood will be placed into conical freezer microtubes in the following manner:
    1. **Tube 1:** 0.1mL of $2x10^5$ cells of final AdHER2 DC vaccine product ***<u>only</u>***
    2. **Tube 2**: 0.1mL of $2x10^5$ cells of final AdHER2 DC vaccine product ***<u>plus</u>*** 0.2mL of heparinized whole blood.
    3. **Tube 3**: 0.2mL of heparinized whole blood ***<u>only</u>***
- Incubate at 37°C for 4 hours.
- Store in -80°C (+/- 10°C) freezer until ready for shipment to Hitachi Chemical
- **Note:** ***specimen should be drawn DAY OF VACCINE before 1pm*** to allow adequate time for specimen processing and four hours incubation by Trepel lab staff.

Multiparameter Flow Cytometric Analysis of Circulating Tumor Cells (CTC) and Immune Cell Subsets: Jane Trepel, DTB

Analysis will be performed by the laboratory of Jane Trepel. CTC cells and immune cell subsets will be identified by mulitparameter flow cytometry. The order of priority for immune subset analysis is T, B, NK, NKT, Tregs, MDSC and dendritic cells.

CTC Analysis: One 10mL lavender top tube
(Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124 when patient schedule permits collection before 1pm.)

Immune Cell Subset Analysis: One 10mL lavender top tubes

(Weeks 0, 8, 16, 24, 32, 40, 48, 60, 76, 100, and 124 when patient schedule permits collection before 1pm.)

Multiparameteric flow cytometry using a Miltenyi Quant flow cytometer. The order of priority for immune subset analysis is T, B, NI, NKT, Tregs, MDSC and dendritic cells.

NKT Cell Analysis: One 10mL lavender top tubes
(Weeks 0, 12 (s/p 3 doses of vaccine), 28 and 48)

Sample Logistics for both Function-Associated mRNAs and CTC and Immune Subsets:

- **Notify the Trepel lab via email** when the clinical sample is scheduled to be drawn:
  - Sunmin Lee (lees@pop.nci.nih.gov)
  - Min-Jung Lee (leemj@mail.nih.gov)
  - Jane Trepel (trepel@helix.nih.gov)
- Label the clinical specimen tubes and *include the study week number*.
- **Note: *specimen should be drawn before 1pm*** to allow adequate time for four hours incubation for the Hitachi clinical specimens.
- **Phone the Trepel lab at 240-760-6330** when the specimen is drawn for pick up by the Trepel lab.
- The laboratory of Jane Trepel where specimens will be processing and cryopreserved is in Bldg.10, Rm. 12C208

Immune and molecular profiling through RNA sequencing*:*

Briefly, patient blood samples collected at baseline and at each time point when vaccine is administered is analyzed via next generation sequencing and RNA sequencing with the aim of identifying potential biologic/immunological correlates of vaccine mediated efficacy, including but not limited to the potential upregulation of checkpoints using advanced bioinformatics tools.

Subcontractor: Novogene Corporation, Inc. 8801 Folsom BLVD, Suite 290, Sacramento, CA 95826 by Midissia Therapeutics, Inc.

Specimen Processing:. 10ml of whole blood will be collected in should be in 2 Tempus RNA tubes at Weeks 0, 8, 16, 24, 30, and 48 and kept frozen at -80°C in Frederick Leidos lab until analysis. If Tempus tube sample is not available, any unused PBMNC frozen aliquots for cellular response evaluation can be used as an alternative.

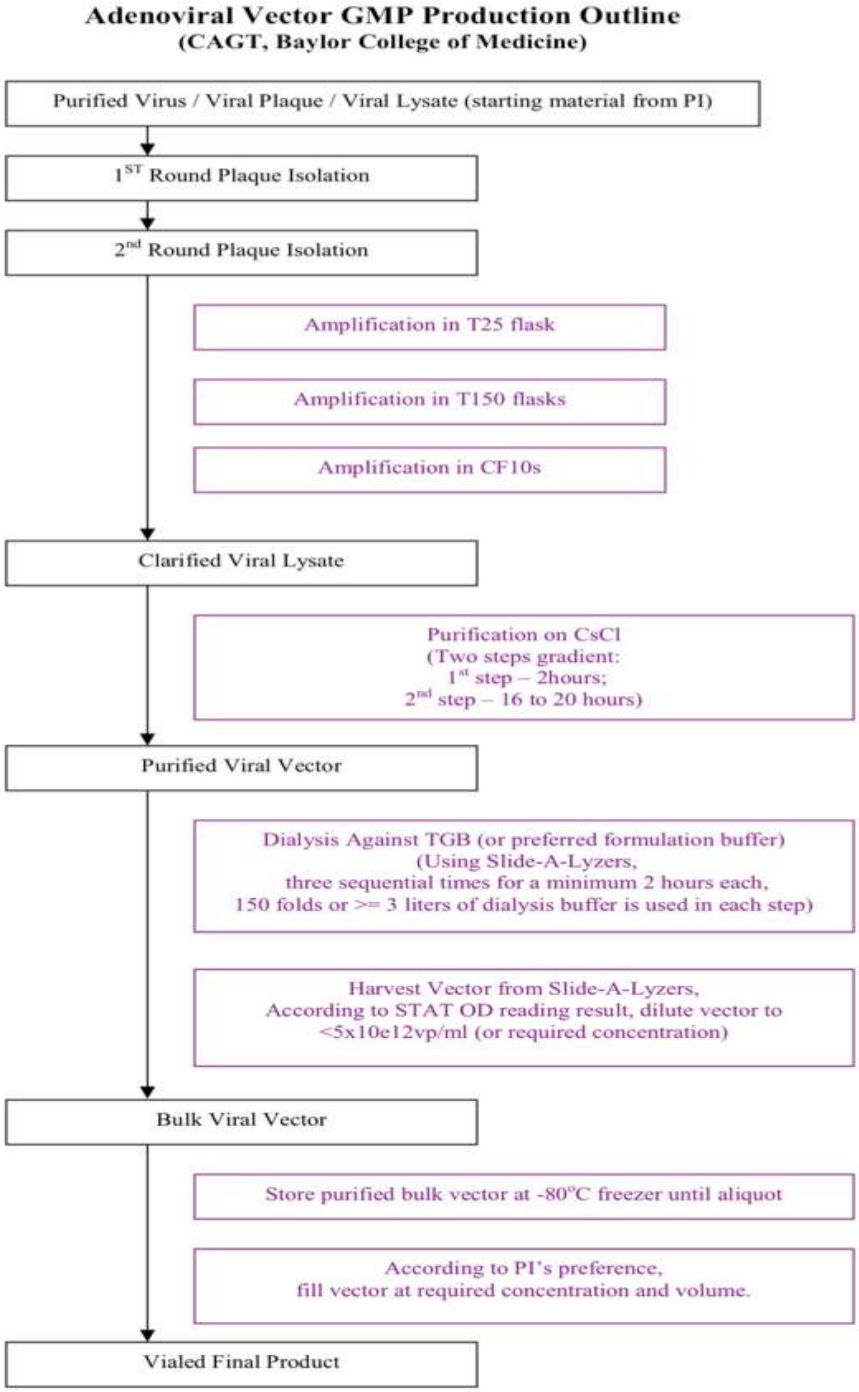
RNAseq will be performed on patient blood samples to provide a comparative analysis of gene expression at baseline and following treatment. This will provide a longitudinal scan of patient response to treatment. To ensure Quality control, RNA quantity and quality are assessed using OD_{260} and $OD_{260/280}$ ratios. RNAseq read quality is also subject to diligence. Each patient sample will have the entire transcriptome read and comparisons to baseline will be made by number of reads at each gene.
Significant changes will be determined by a threshold p-value and a list of Differentially Expressed Genes (DEG) will then be assembled. DEG gene lists will then be used as input for *post-hoc* analysis. *Differential Expression analysis* provides data on the number of genes whose expression changes over the course of treatment and is presented by Venn diagram or volcano plot. *Gene Set Enrichment Analysis* uses the lists of differentially expressed genes (DEG) and compares them to sets of curated gene sets in 4 databases.

- Gene Ontogeny (GO): a curated gene set associated with normal cell processes, such as: *regulation of cell motility.*
- Disease Ontogeny (DO): a curated gene set associated with disease processes, such as: *thrombocytosis*
- Kyoto Encyclopedia of Genes and Genomes (KEGG): a curated gene set associated with signal transduction pathways.
- Reactome: Another database of signal transduction nodes.

DEG lists from patients will be used to identify genes associated with signal transduction pathways, cellular processes and disease states to identify individual genes or groups of genes associated with a gene set of interest. Those genes whose expression patterns change predictably with clinical response will ultimately be used as a biologic/immunological correlate of vaccine mediated efficacy.

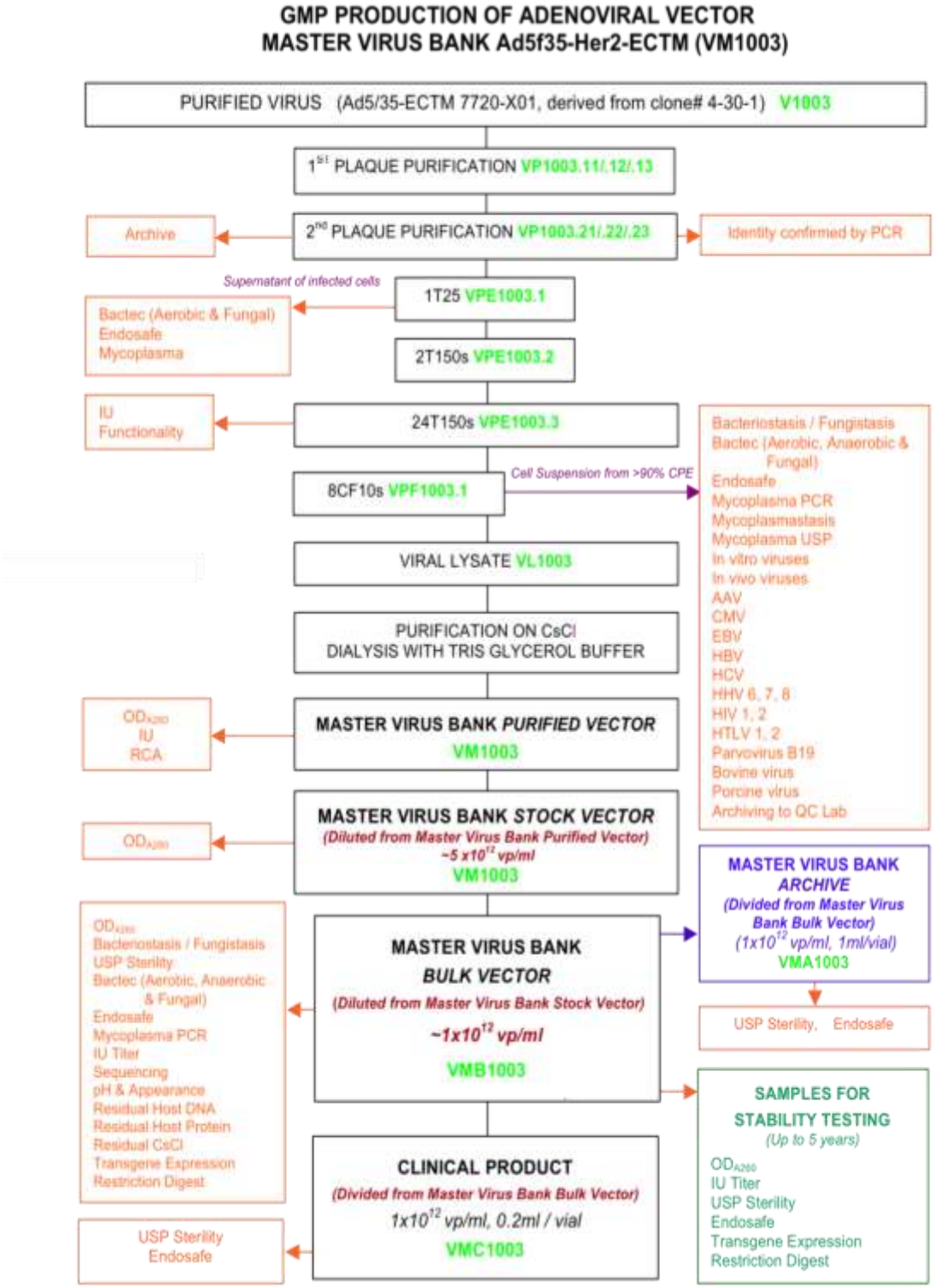
15.10 APPENDIX 10AD5F35HER2ECTM (ADHER2) VECTOR MANUFACTURING

GMP Production Outline (1 of 2)

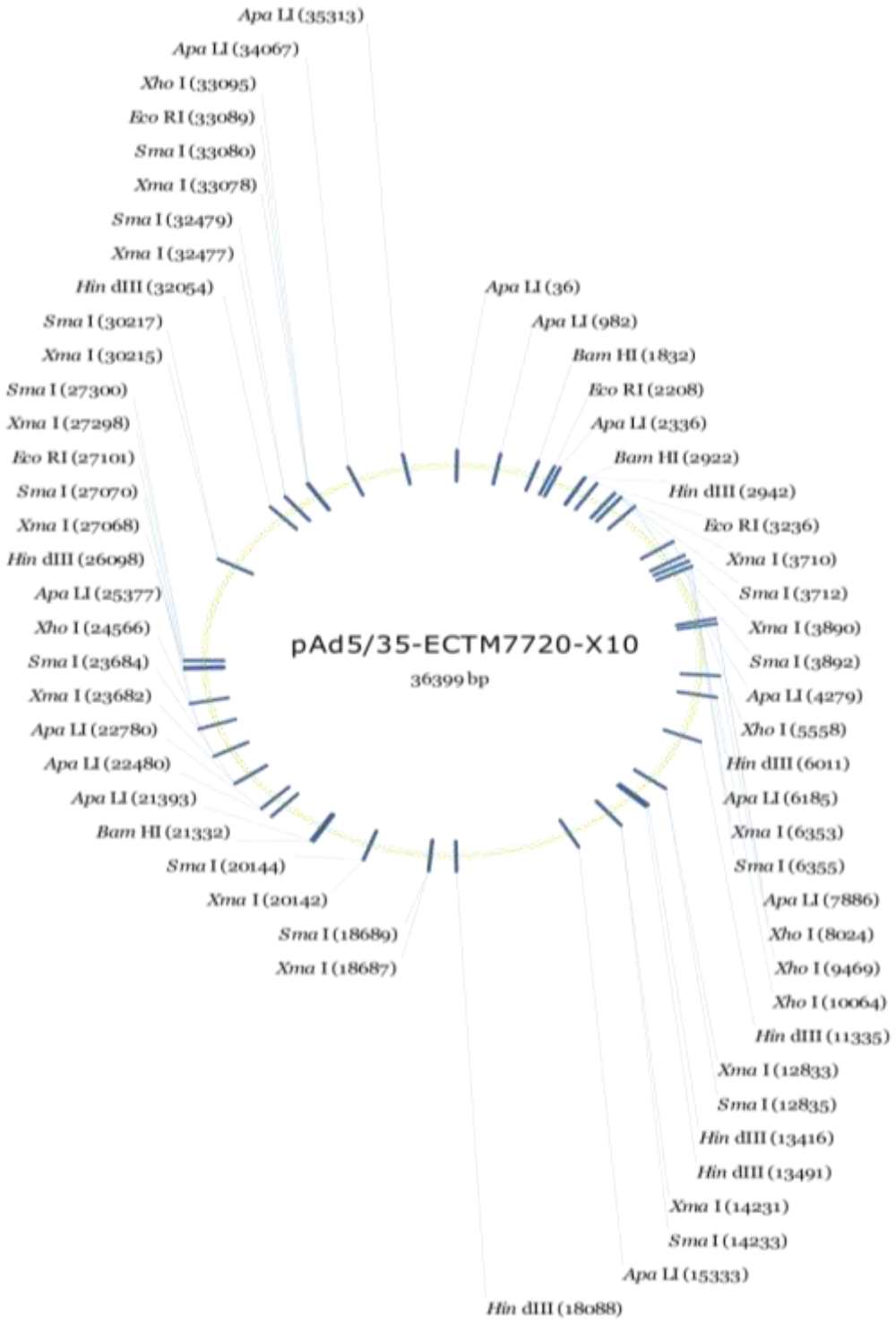


Ad5f35HER2ECTM (AdHER2) Detailed Vector Manufacturing (2 of 2)

QC Highlighted in Orange



15.11 APPENDIX 11MAP OF THE AD5F35HER2ECTM (ADHER2) VECTOR



15.12 APPENDIX 12 ADHER2 DENDRITIC CELL VACCINE CANCER TREATMENT HISTORY

Brief Cancer History:

Cancer Diagnosis	
Date of Diagnosis	
Stage of Cancer	

Surgical Procedures:

Operative Procedure	**Date**	**Comments**

Prior Chemotherapy:

Agent/Regimen	**Date/Duration**	**Comment/Reason for D/C**

Prior HER2-Targeted Therapies: (trastuzumab, lapatinib, pertuzumab)

Agent	Date/Duration	Comment/Reason for D/C	
HISTORY OF HER2 THERAPY RELATED CARDIAC DYSFUNCTION (If yes document name of agent and describe toxicity and intervention)		YES	NO

PATIENT IS NOT ELIGIBLE FOR THIS STUDY IF POSITIVE FOR HER2-RELATED CARDIAC TOXICITY

Prior Radiation Therapy

Therapy Type	Date/Duration	Comment

Pathology: HER2/neu expression

HER2 Detection Method	Negative	Equivocal	Positive
Vysis FISH Ratio (Measures HER2 oncogene)	Ratio < 1.8	Ratio 1. 8 – < 2.2	Ratio > 2.2
IHC (Measures HER2 protein)	0	1+ - 2+	3+

Part I and Part II Inclusion

Part I: Adults ≥ 18 with recurrent or progressive, metastatic solid tumors characterized by some HER2/neu expression that have failed standard therapies with known benefit but for whom trastuzumab is not clinically indicated.

Part II: Breast cancer patients with 3+ HER2/neu expression by IHC or a positive or an equivocal or positive FISH result with prior exposure to trastuzumab, lapatinib or pertuzumab.

Part I or Part II	HER2 Detection Method	Expression	Date of Collection	Received from NIH or Outside Lab
	Vysis FISH Ratio			
	IHC			

Additional Comments:

Person Completing This Form:_____

15.13 APPENDIX 13 PATIENT RECRUITING MATERIALS

In accordance with standard IRB regulations requiring approval of patient recruitment materials, The following script is for an informational video that will be posted to the NCI YouTube Network sponsored by the NCI Office of Communications at: www.youtube.com/ncigov. The informational video will feature Dr. Lauren Wood, the former Principal Investigator of the AdHER2 DC vaccine study and will be approximately 2-4 minutes in duration. The video link will be embedded in all standard patient recruitment materials sent out by the NCI CCR Office of Patient Outreach and Recruitment as well as the NIH Clinical Center Office of Communications, Patient Recruitment and Public Liaison. This informational video is a novel patient recruitment tool that is being piloted with this study and will be assessed for its impact on rates of study accrual.

AdHER2 DC Vaccine Informational Video Script

Hello. My name is Dr. Lauren Wood and I am the Principal Investigator of a new therapeutic cancer vaccine study that is being conducted at the NIH Clinical Center in Bethesda, MD. Therapeutic cancer vaccines are given to patients that already have cancer and are designed to train the immune system to better recognize and kill cancer cells. The hope is that once trained, cells of the immune system may be able to seek out and destroy cancer cells in areas of the body where chemotherapy drugs and radiation may not. Therapeutic vaccines may also continue to have an anti-tumor effect that persists even after the vaccine is no longer being given.

The clinical trial we are conducting is studying a cancer vaccine that is being given to humans for the first time. The science behind the trial is based on animal models that showed that a single dose of a mouse vaccine, similar to the human vaccine being used in this study, could cure large established tumors in mice.

The cancer vaccine is designed to target a tumor protein called HER2. The HER2 protein makes tumors more aggressive and more likely to spread. As a result, patients with HER2 positive tumors often don't do as well. One of the most common tumors associated with HER2 expression is breast cancer, although only about 25 percent of women with breast cancer have HER2 positive tumors. In addition, there are other tumor types that express the HER2 protein, including bladder, colon, lung, kidney, ovarian, and prostate cancers.

Because the HER2 protein makes tumors more aggressive, a number of different drugs targeting it have been developed to treat cancer. One of the first drugs was trastuzumab, also known by the brand name Herceptin. It is approved for the treatment of breast and stomach cancer. As a monoclonal antibody, trastuzumab recognizes a distinct, small portion of the HER2 protein.

Although it's been shown to benefit patients through multiple studies, some patients do not respond to treatment, some patients become resistant to treatment and still others don't have high enough levels of the HER2 protein in their tumors to benefit from trastuzumab treatment. There are also other drugs that are licensed or in development that target HER2 by different mechanisms.

The cancer vaccine in this trial uses cells from the patient's own immune system to generate a custom-made vaccine specific for each patient. The immune system cells we use are called dendritic cells. A "dead" virus, that is not able to make copies of itself, is used to deliver the HER2 genetic material inside the dendritic cells. This causes cells to express the HER2 protein on their surface and the dednritic cells are then given back to the patient as a vaccine. The hope is that this custom-made cancer vaccine will be able to stimulate the patient's own immune system to make their own antibodies to HER2, and that these antibodies will recognize multiple sites of the HER2 protein, rather than just one limited site like trastuzumab.

The primary goal of this study is to determine whether or not the vaccine is safe in human beings and determine if there is an optimal dose to give. We also want to know whether or not the vaccine stimulates the immune system to produce antibodies and whether these antibodies will help slow the growth of or possibly even shrink tumors. A very small percentage of people who are treated with trastuzumab develop cardiac toxicity. We don't have a good understanding of what causes the changes in heart function and we don't know whether or not the HER2 vaccine we are testing will have any adverse effects on the heart. It is why if you choose to participate in this study, you will be monitored very closely during the study and for some time even after your last dose of vaccine. Importantly, there have been multiple clinical trials of HER2 cancer vaccines and to date, none of them have been associated with the development of problems in heart function.

Initially we are seeking patients who have recurrent disease with tumors that have some level of HER2 expression but who have never been treated with trastuzumab or any other HER2-directed therapies and do not qualify to be treated with these therapies. Multiple tumor types are allowed. Once we have established safety of the vaccine in this population, we will evaluate the vaccine in patients with breast cancer that have progressed despite treatment with trastuzumab and other HER2 therapies.

If you would like further information about this clinical trial, please call 888-XXX-YYYY to speak with a research referral coordinator. Again, my name is Dr. Lauren Wood of the NCI Vaccine Branch in Bethesda, MD. Thank you for taking the time to watch this video and for your interest in our clinical study.

Current run time with normal voice cadence and pace: 5 minutes 8 seconds.

Further script editing and reduction in duration may occur following review by NCI Communications.

If successful, this Principal Investigator informational video link could be replicated for multiple other clinical trials being conducted by the NCI CCR, the Clinical Center and other NIH institutes.

Contact Information of Key Partners for Proposed Pilot Video Recruitment Tool:

James Alexander
Public Affairs Specialist
Office of Partnerships and Dissemination Initiatives
Office of Communications and Education
National Cancer Institute, NIH
6116 Executive Blvd, oom 4117
Bethesda, MD 20892-8351
Phone: 301-402-4917
Email: alexandj@mail.nih.gov

Dinora Dominguez
Chief, Patient Recruitment and Public Liaison Section
Office of Communications, Patient Recruitment and Public Liaison
NIH Clinical Center
National Institutes of Health
Department of Health and Human Services
10 Cloister Ct., Bldg 61
Bethesda, MD 20892-4754
Direct Line: 301-402-6072
Fax: 301-480-8640
Toll-Free Patient Referral Line:
1-800-411-1222
TTY: 1-866-411-1010
Internet: http://www.cc.nih.gov
Study Information: http://clinicalstudies.info.nih.gov
Twitter: NIHClinicalCntr
Facebook: www.facebook.com/NIHClinicalCenter
YouTube: www.youtube.com/user/NIHClinicalCenter
Audio Podcasts: clinicalcenter.nih.gov/podcast

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Office of the Clinical Director

Center for Cancer Research, NCI
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mcmulles@mail.nih.gov

Hoyoung Maeng, M.D.
Vaccine Branch, NCI
Bldg. 10, Rm. 3B37
10 Center Drive, MSC 1578
Bethesda, MD 20892-1578
Phone: 240-781-3253
FAX: 301-480-8514
Email: hoyoung.maeng@nih.gov

13-C-0016 INFORMATIONAL VIDEO WEBLINKS:

Healthcare Provider Informaional Video (run time 3:21):

https://youtu.be/TxXsflkDfYQ

Patient Informational Video (run time 3:25):

https://youtu.be/ejscyiHe5t0

These informational videos are also posted on the following web sites:

Dr. Wood's CCR Bethesda Trials Investigator Profiles web site:

http://ccr.cancer.gov/staff/staff.asp?profileid=5570

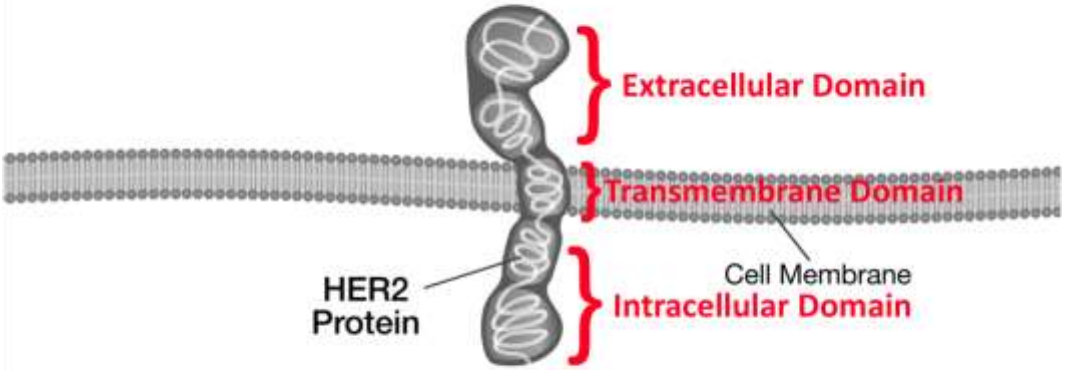
In the CCR News web site Multimedia section:

http://ccr.cancer.gov/news/InTheirOwnWords/Default.aspx

Additional informational materials provided to patients: 13-C-0016 Science in a Snapshot Slide Deck

NOTE: These "snap shots" and their content are taken directly from the informational videos that were approved by the NCI IRB on 02/25/13.

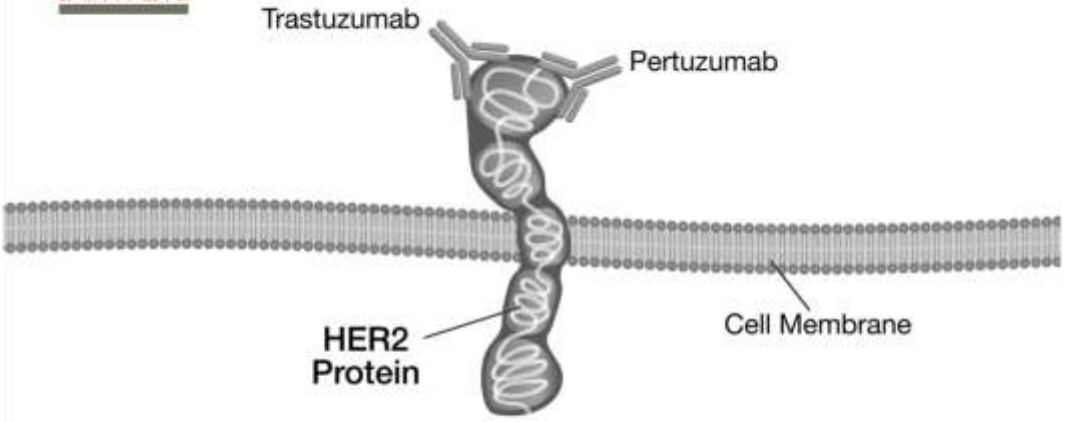
Science In A Snapshot: The AdHER2 DC Vaccine NCI Protocol 13-C-0016



- The HER2 protein is over-expressed in breast and many other types of cancers.
- It sends signals to tumor cells to make them grow and prevents them from dying.
- Tumors that over-express HER2 are associated with more aggressive disease, higher recurrence rates and reduced survival rates.

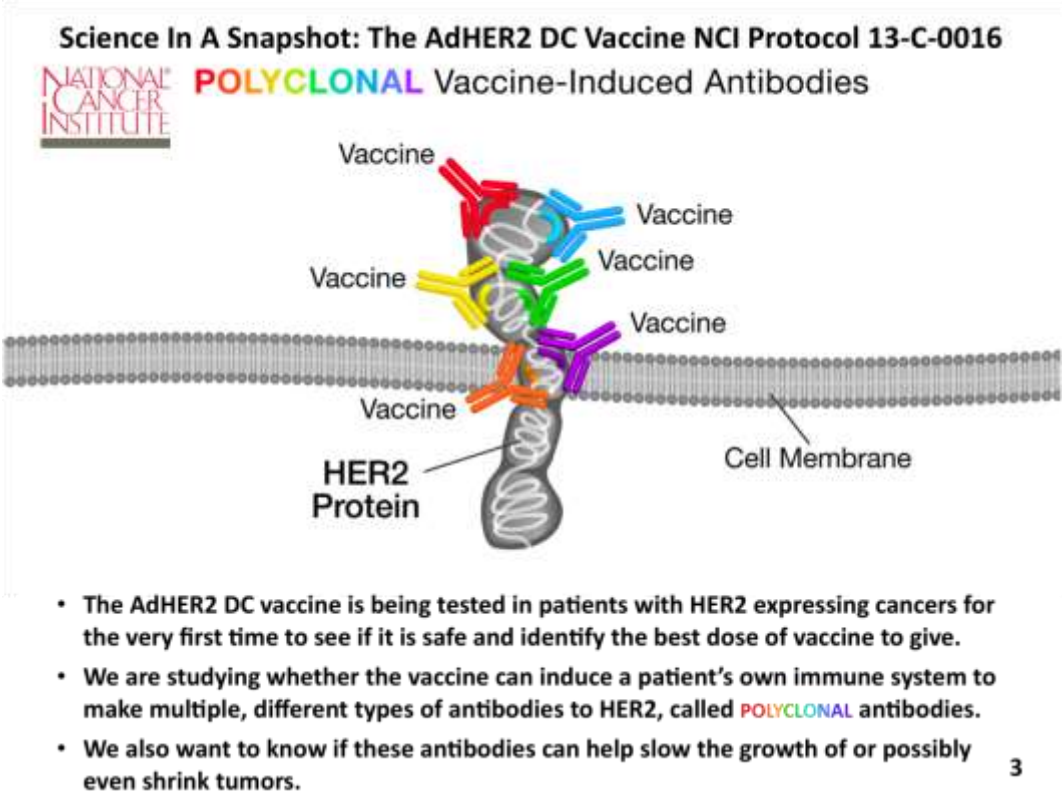
1

Science In A Snapshot: The AdHER2 DC Vaccine NCI Protocol 13-C-0016



- Trastuzumab (Herceptin®) and pertuzumab (PERJETA™) are monoclonal antibodies that each recognize, a distinct, small portion of the HER2 protein.
- These monoclonal antibodies have to be given repeatedly to have an effect.
- Despite their effectiveness in some cancers, trastuzumab and pertuzumab don't work for everyone and even when they are effective, they may stop working after some time.

2



Additional materials for posting on social media sites in accordane with NIH Social Media Policy and coordinated by the NIH Clinical Center Patient Recruitment and Public Liaison Office (Dinora Dominguez) and the NCI Office of Communications and Education (James Alexander):

13-C-0016 Social Media Site Language Requiring IRB Approval

NIH Clinical Center Atrium Screen

NIH vaccine researcher is studying individuals with bladder, colon, lung, ovarian, prostate, and kidney cancer. Watch Dr. Lauren Wood's videos to learn how vaccines are involved in cancer research.

More information

Video for Patients:

https://youtu.be/ejscyiHe5t0

Video for Healthcare Providers

https://youtu.be/TxXsflkDfYQ

Homepage slide

Phase 1 trial for breast, bladder, colon, lung, ovarian, prostate, kidney and other cancers that express HER2. Watch video to learn about vaccines in cancer research.

More information

Video for Patients:

https://youtu.be/ejscyiHe5t0

Twitter, GovDelivery,

135 characters- NIH vaccine researcher studying breast, bladder, colon, lung, ovarian, and other cancers. Watch videos to learn how vaccines are involved in cancer research.

OR

129 characters- Watch videos for a cancer vaccine clinical trial for patients with HER2+ solid tumors including breast, bladder, colon, lung, ovarian and other cancers.

OR

131 characters- NIH vaccine researcher is studying individuals with bladder, colon, lung, ovarian, prostate, and kidney cancer. Watch Dr. Lauren Wood's vaccine videos. *(this is more plain language and patients will be able to quickly relate to one of the following cancers)*

Facebook:

Dr. Hoyoung Maeng, a NCI vaccine-researcher, recently became the PI of a Phase I trial to evaluate the safety and immune response to a therapeutic cancer vaccine designed to stimulate the immune system to recognize the HER2/neu gene in patients with solid tumors including breast, bladder, colon, lung, ovarian, prostate and other cancers.

Video for Patients:

https://youtu.be/ejscyiHe5t0

Video for Healthcare Providers:

https://youtu.be/TxXsflkDfYQ