SUMMARY OF CHANGES

For Protocol Amendment #7 to #8

HCC #: 12-110

Old Protocol Date: 03-18-2016 New Protocol Date: 02-23-2018

#	Section	Page(s)	Change
1.	Title page and header	All	Updated version # and/or date, Change in PI from Zeh to Bartlett

TITLE: A Phase 1/2 Trial Evaluating αDC1 Vaccines Combined with Tumor-Selective Chemokine Modulation as Adjuvant Therapy after Surgical Resection of Peritoneal Surface Malignancies

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Investigational Agent:	Alpha-type-1 Dendritic Cell Vaccine and Rintatolimod
Commercial Agent:	Interferon-alpha and Celecoxib

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SCHEMA

This trial will evaluate the effectiveness of autologous alpha-type-1 polarized dendritic cell (αDC1) vaccines (patients' autologous αDC1s loaded with autologous tumor material), combined with a systemic chemokine modulation regimen [CKM; intravenous rintatolimod (TLR3 ligand, a derivative of Poly-I:C) + intravenous interferon-alpha + oral celecoxib] as adjuvant therapy, after cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), in patients with peritoneal surface malignancies (PSM), including but not limited to malignant peritoneal mesothelioma and peritoneal carcinomatosis (PC) of appendiceal and colorectal origin.

All patients judged to have peritoneal surface malignancy and considered able to be cytoreduced to Peritoneal Cancer Index (PCI) Completeness of Cytoreduction (CC) score of 1 or less will undergo CRS + HIPEC. Postoperative immunotherapy will start upon recovery from surgery but no sooner than 4 weeks post CRS + HIPEC.

The Phase 2 immunotherapy regimen will include 1 priming dose of the αDC1 vaccine and 3 booster doses of the αDC1 vaccine combined with CKM. Each dose of vaccine, whether priming or booster, will include 1 intranodal (3M cells) and 1 intradermal (3M cells) injection. Phase 1 includes only the priming vaccine dose and 1 cycle of a booster dose + CKM.

Each booster vaccine dose (booster cycles 1-3) will be followed by 4-days of systemic CKM which is comprised of intravenous (IV) IFNα (dose-escalation in Phase 1 only: 5-20 MU/m^2) once a day for 4 days; IV rintatolimod (short-half-life TLR3 ligand) 200 mg on Wednesday and Friday only of the CKM regimen; and oral celecoxib 200 mg twice a day for all 5 days.

In order to avoid overlap between experimental immunotherapy and potential adjuvant chemotherapy (which can be clinically indicated as a part of standard care in a subset of patients), the experimental treatment may be interrupted after each cycle to allow adjuvant chemotherapy that may be done for each patient's clinical care, not as part of this research study. If clinically indicated any cycle of adjuvant chemotherapy may start a minimum of 5 days after completion) of a cycle of immunotherapy and recovery form any potential side effects. The following cycle of immunotherapy may start at least 5 days after the completion of chemotherapy and after recovery from acute side effects of chemotherapy.

Timing of the experimental treatment:

- Priming cycle (vaccine alone): 6 to 16 weeks after surgery (target: 8 weeks)
- Booster cycle 1 (vaccine plus CKM): 4 to 12 weeks after priming cycle (target: 4 weeks)
- Booster cycle 2 (vaccine plus CKM):: 4 to 12 weeks after booster cycle 1 (target: 4 weeks)
- Booster cycle 3 (vaccine plus CKM):: 4 to 12 weeks after booster cycle 2 (target: 4 weeks)

The primary endpoint will be time to tumor progression/recurrence on CT or MR imaging. Secondary endpoints will be immunologic response markers.

The schemas for the trial are located in Sections 5.1 and 5.2.

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LIST OF ABBREVIATIONS

ACE inhibitor	angiotensin-converting-enzyme inhibitor
AE	Adverse event
ALP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase
BUN	Blood urea nitrogen
cc	cubic centimeters
CC score	Completeness of Cytoreduction score
CEA	Carcinoembryonic antigen
cGMP	current good manufacturing practice
CKM	chemokine modulation
CRS	Clinical Research Services
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
CTLs	Cytotoxic T-Lymphocyte
CTRC	Clinical & Translational Research Center
DLT	Dose-limiting toxicity
DSMB/DSMC	Data Safety Monitoring Board/ Data Safety Monitoring Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
GM-CSF	granulocyte-macrophage colony stimulating factor
HSA	human serum albumin
IB	Investigator's brochure
IFNα	interferon alpha
ILCI	intensive locoregional chemotherapy and immunotherapy
IN	intranodal
IND	Investigational new drug
IP	intraperitoneal
IRB	Institutional Review Board
ITC	Immunotransplantation Center
IV	intravenous
MDSC	myeloid-derived suppressor cells
Mg	milligrams
mL	milliliter
MRI	Magnetic resonance imaging
MTD	maximum tolerated dose
MU	million units

NCI	National Cancer Institute
NK cells	natural killer cells
NSAID	non-steroidal anti-inflammatory drug
OS	Overall survival
OvCa	ovarian cancer
PBMC	Peripheral blood mononuclear cell
PET	Positron emission tomography
PFI	progression free interval
RP2D	Recommended Phase 2 dose
PS	Performance status
PTT	partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
SAE	Serious adverse event
SOC	Standard of care
TILs	tumor infiltrating lymphocytes
TLR	toll-like receptor
TTP	Time to progression
ULN	Upper limit of normal
UPCI	University of Pittsburgh Cancer Institute
USP	United States Pharmacopeia
UV	ultraviolet
αDC	alpha dendritic cell

1. OBJECTIVES

1.1 Primary Objectives

Preliminary data from our laboratory indicate that autologous antigen-loaded αDC1 vaccines induce highly-effective tumor-specific $CD8^+$ CTLs that express defined chemokine receptors, CCR5 and CXCR3.[1-3] We have also demonstrated that IFNα and poly-I:C enhance local production of relevant chemokines CCL5/RANTES and CXCL10/IP10 that are able to attract the CCR5- and CXCR3-expressing CTLs selectively to tumor lesions, rather than in marginal healthy tissues. Furthermore, the above factors suppress local production of undesirable chemokines (CCL22) that attract immunosuppressive T_{reg} cells.[4] In addition, studies have demonstrated a correlation between the density of infiltrating effector T cells and long-term outcomes in malignant peritoneal mesothelioma[5,6] and colorectal cancer[7,8].

In this Phase 1/2 clinical trial, we will test the effectiveness of the treatment of patients with peritoneal surface malignancies, including but not limited to malignant peritoneal mesothelioma (MPM) and peritoneal carcinomatosis (PC) from appendiceal and colorectal primary tumors, using autologous αDC1 vaccines combined with a systemic chemokine modulation regimen, consisting of celecoxib, IFNα, and TLR3 ligand as a component of adjuvant therapy. We anticipate that the resulting tumor-selective modulation of the peritoneal chemokine environment will promote the entry of spontaneously-occurring and αDC1-induced effector-type T (*eff*) cells into tumor, and limit the influx of undesirable regulatory-type T (*reg*) cells.

Our primary objective is to test whether our combined immunotherapy regimen (αDC1 vaccines + CKM) is safe and whether it can prolong time-to-progression (TTP) in patients with peritoneal surface malignancies after optimal CRS + HIPEC with or without adjuvant chemotherapy.

1.2 Secondary Objectives

- To estimate overall survival (OS).
- Perform correlative studies to characterize sequential immune response changes by quantitative measures of immune cellular phenotypes and chemokine patterns in peripheral blood. These results will be correlated with TTP, PFS, and OS in attempt to predictive markers of the clinical benefit.

2. BACKGROUND

2.1 Tumor Immunogenicity in Malignant Peritoneal Mesothelioma

Malignant mesothelioma is a rare but aggressive primary malignancy that almost exclusively develops from the serosal lining of the pleural (incidence ~ 2000 cases/year) or peritoneal cavities (incidence ~ 250-500 cases per year).[9] Malignant peritoneal mesothelioma is an aggressive loco-regionally invasive disease that rarely involves lymph nodes (5-10%) or metastasizes extra-abdominally (3-5%). Compressive symptoms occur due to progressive accumulation of tumor and ascites in the abdomen, which leads to organ-dysfunction, morbidity, and eventual mortality.[10] Prognostic factors for improved overall and disease-free survival

include epithelioid histology, lack of lymph node disease, small nuclear size (I/II), adequate CRS (CC-0/CC-1), low mitotic count ≤ 5 mitoses/50 HPF, age ≤ 60 years, female gender, prior surgical debulking, lack of deep tissue invasion, hyperthermic intraperitoneal chemoperfusion (HIPEC), and early stage of disease (Stage I/II).[11,12]

Traditional systemic chemotherapy and palliative surgical procedures provide minimal benefit over the natural history of untreated disease, with median survival of 12 months and rare long-term survivors. The most effective systemic chemotherapy regimen available for unresectable disease, combination cisplatin with 3rd generation antifolates pemetrexed and raltitrexed, demonstrates best response rates of up to 40%, mortality risk reduction of 10% at 1 year and corresponding improvement in survival of 6-8 weeks.[13] Recent experience with aggressive cytoreductive surgery in combination with HIPEC has demonstrated median survival of 36 to 92 months and 29% to 59% 5 year survival in retrospective analyses.[14] However, disease recurrence is common and survival remains especially poor for high-risk patients with non-epithelioid histology, high mitotic index and incompletely resected tumors, despite mulimodality therapeutic strategies including neoadjuvant or adjuvant chemotherapy protocols in addition to cytoreductive surgery and intraperitoneal chemotherapy. This underscores the need for more effective targeted therapies, especially in patients with high-risk recurrent malignant peritoneal mesothelioma.

Studies have shown that patients with malignant pleural mesothelioma who demonstrate robust anti-tumor immune responses survive longer. Increased CD8+ tumor-infiltrating lymphocytes (TILs) are associated with lower incidence/burden of mediastinal lymphadenopathy, higher numbers of apoptotic tumor cells in tumor tissue, as well as improved progression-free and overall survival after surgical resection, with or without neoadjuvant chemotherapy. This suggests that stimulation of CD8+ TILs is a potential therapeutic strategy. In fact, patients receiving neoadjuvant chemotherapy with cisplatin/pemetrexed combination demonstrate higher CD8+ TILs than those receiving other chemotherapy regimens, lending credence to this being the most efficacious regimen in randomized trials.[5,6] However, the immune cell population of mesothelioma strongly favors a suppressive environment, with heavy infiltration of CD4+CD25+Foxp3 regulatory T cells (T_{regs}), and an absence of CD1α+ DCs, which might explain the lack of tumor kill despite high levels of effector T cells. This immunosuppressive environment must be modulated to improve immunotherapeutic efficacy.[15]

Intra-peritoneal delivery of tumor-lysate pulsed DCs prior to intra-peritoneal tumor implantation of mouse mesothelioma cell line AB1 prevents tumor engraftment, halts mesothelioma progression and provides long-term protection against tumor re-challenge. In an adjuvant setting, this immunotherapy demonstrates a better outcome when delivered early after AB1 tumor implantation, when tumor burden is less. Mice receiving DC vaccine have higher AB1 cell lysis (chromium release assay) and higher IFNγ production from splenocytes (ELISPOT assay), suggesting an overall increased CTL activity. Adoptive transfer of CD8+ T cells, isolated from splenocytes of immunized mice, into non-immunized AB1 tumor bearing mice improves survival.[16] A small Phase 1 clinical trial (10 patients) in patients with mesothelioma was performed to assess toxicity and feasibility of clinical grade DC vaccine immunotherapy and tumor response to immunotherapy. DCs were pulsed with autologous tumor lysate, matured ex-vivo, and then administered intradermally and intravenously to select malignant pleural mesothelioma patients with epithelioid histology that demonstrated partial response or stable disease following CRS + cisplatin chemotherapy. DC immunotherapy was well tolerated and

appeared to elicit an immunologic response (positive DTH skin test, increased inflammatory cells in skin biopsies at site of vaccination, increased antitumor T cells in blood) and some clinical response by RECIST (of 10 patients, 6 had progression, 1 had stable and 3 had partial response), however no survival benefit was noted.[17] Recent evidence indicates that chemotherapeutic agents can induce specific immune responses, thereby improving therapeutic outcomes. Chemotherapy induced dead tumor cells may release "danger signals" that induce DC maturation with subsequent CTL activation. In a murine model of mesothelioma, gemcitabine has been shown to increase antigen presentation to CD8+ T cells. Vaccination of these tumor-bearing mice following gemcitabine therapy resulted in a marked decrease in tumor growth and increased survival compared with vaccination without gemcitabine.[18] Similarly, certain cytotoxic drugs like cisplatin have been shown to increase the sensitivity of tumor cells to immune-mediated attack.[19]

2.2 Tumor Immunogenicity in Colorectal Adenocarcinoma with Peritoneal Carcinomatosis

Cancer of the colon and rectum (colorectal cancer) is the third leading site of cancer diagnosed annually in the United States and the most common form of gastrointestinal malignancy. It accounts for approximately 150,000 new cases annually. Isolated peritoneal carcinomatosis in the setting of colorectal adenocarcinoma occurs synchronously in 8% and metachronously in 25% of patients. Median survival without therapy is 6 months, while systemic chemotherapy improves median survival in unresectable patients to 12.6 months, with rare 5 year survivors.[20] Aggressive CRS + PIC has demonstrated median survival of 30-60 months and 20-50% 5 year overall survival rates, with particular benefit seen in patients undergoing complete CRS (CC-0/CC-1) for low volume disease (low peritoneal carcinomatosis index; PCI).[21]

Signs of an immune response, including increased mRNA levels for products of TH1 effector cells, markers of T cell migration, activation and differentiation, and CD8+ T cell infiltration within colorectal cancers are associated with the absence of early metastasis; increased density of CD45RO+ memory T cells and improved OS.[8] Similarly, the type, location and density of TILs in colorectal cancer patients are strong predictors of survival. Co-expression of genes for cytotoxicity and TH1 adaptive immunity predicts patient survival independent of metastatic status.[7] A meta-analysis of studies involving active specific immunotherapy (ASI) in patients with minimal residual colorectal cancer after R0 resection (6 studies, 1375 patients) and patients with advanced unresectable colorectal cancer (43 studies, 656 patients) was recently published. ASI included autologous tumor cell vaccines, defined tumor-protein vaccines, monoclonal antibodies, peptide vaccines, viral vector vaccines, DC vaccine and naked DNA vaccines. The authors demonstrated improved disease-free survival for stage II disease and improved disease-free/overall survival for stage III disease in the setting of minimal residual colorectal cancer, while objective response rate (complete response + partial response; 1.6%) and clinical benefit rate (complete response + partial response + stable disease; 26%) were low in the setting of advanced unresectable colorectal cancer. They hypothesized that ASI may target cancer stem cells or micrometastases that are responsible for cancer recurrence/metastasis and are resistant to conventional chemotherapy.[22] Autologous tumor lysate-pulsed DC vaccine administered intranodally to patients with colorectal metastasis after complete surgical resection induced tumor-specific T-cell proliferation or IFNγ secretory response in 63% of patients and a DTH response to autologous tumor cells in 61% of patients, with subsequent improved recurrence-free

survival at 5 years in these patients.[23] A pilot study evaluating the immunogenic effect of chemotherapy by concurrent CEA-peptide pulsed DCs vaccination and systemic oxaliplatin/capecitabine as adjuvant therapy in stage III colorectal cancer patients after complete resection demonstrated stable levels of peripheral blood mononuclear cells during chemotherapy, CEA-peptide-specific T-cell responses and higher production of IFNγ and IL-2 upon co-culture with CEA-loaded target cells.[24] In a Phase 1/2 trial using DCs transfected with RNA encoding CEA to vaccinate patients after complete resection of colorectal liver metastasis, evidence of immune response in skin biopsies of DC injection sites and CEA-specific T-cell activity in peripheral blood was demonstrated.[25]

2.3 Tumor Immunogenicity in Appendiceal Adenocarcinoma with Peritoneal Carcinomatosis

Appendiceal neoplasms are predominantly mucinous and frequently lead to peritoneal carcinomatosis. Classically, they are clinically characterized by progressive, intra-peritoneal dissemination of mucin, referred to as pseudomyxoma peritonei. These tumors comprise 1% of all colorectal cancers, with an annual incidence of 1500 cases in the United States. Their clinical course is primarily determined by the compressive effects of mucin accumulation and tissue invasion by neoplastic epithelial cells. Aggressive cytoreductive surgery with perioperative intra-peritoneal chemotherapy is considered the standard of care for managing these tumors.[26] Multiple large retrospective studies have demonstrated markedly improved patient survival, decreased tumor recurrence, longer time to disease progression and less frequent re-operative interventions with this radical approach, when compared to historical serial debulking procedures or palliative systemic chemotherapy. Unfortunately, these tumors frequently recur, traditional systemic chemotherapy agents show minimal benefit, and targeted therapies are lacking. Multivariate Cox-regression analyses have consistently demonstrated incomplete cytoreductive surgery (CC-2/3) and non-DPAM histology to be the most consistent negative predictors of survival in patients undergoing CRS and PIC.[27] This underscores the need for more effective targeted therapies, especially in patients with high-risk appendiceal cancers with peritoneal carcinomatosis/pseudomyxoma peritonei.

Research related to the immunogenicity of these tumors and the application of immunotherapy alone or in conjunction with systemic chemotherapy is lacking for this malignancy, but is expected to be similar to colorectal cancer, based on the tissue of tumor origin and similar location of the disease.

2.4 Dendritic cells in tumor immunology

Several lines of evidence derived from both murine studies and human clinical trials suggest that cancer can be susceptible to immune-based therapies. Studies using dendritic cells to stimulate tumor-specific immune responses have been particularly encouraging.[28-30] DCs are the most potent antigen presenting cells (APCs), capable of efficiently internalizing and presenting antigen in the context of co-stimulatory signals and cytokines essential to the induction of effective long-lasting T-cell mediated immunity. Animal models have demonstrated that DCs, pulsed ex vivo with defined tumor antigens or material derived from tumor cells, can induce protective tumor-specific immune responses and are capable of mediating the regression of established disease.[31] Human clinical trials, including our own, demonstrate that DCs pulsed

with defined tumor-relevant antigenic peptides, or, alternatively, with tumor cell lysates or apoptotic bodies, can induce tumor-specific immune responses and even occasional complete tumor regression in late stage cancer patients.[29]

Several competing approaches to DC therapy are currently being evaluated in clinical trials. In this context, two emerging issues appear to be critical for the development of "optimal" DC-based immunization strategies. First, defining a strategy for the delivery of tumor antigens that facilitates efficient DC presentation of a broad range of class I- and class II-restricted epitopes appears to be critical to the induction of effective antigen-specific T-cell immunity. Second, recent results suggest that in order to induce tumor regression and promote long-term disease-free status, DC-based strategies need to drive in vivo expansion and maintenance of Th1/Tc1-type T cells (both $CD8^+$ and $CD4^+$) effector function.

Human DCs may be readily obtained in large numbers from peripheral blood by short term in vitro culture in media containing interleukin-4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF).[32] In particular, for the purposes of this protocol, we can anticipate a yield of above 5 x 10^6 DCs per 200 mL of patient peripheral blood after 7 days of in vitro culture, where a single vaccination would consist of 3 x 10^5, or 3 x 10^6 antigen-loaded DCs, and the remaining cells will be used for vaccine testing, quality control, immunomonitoring, and the development of in vitro correlates of the in vivo (immunologic and potentially therapeutic) efficacy of DC.

2.5 Type-1 polarized DC (αDC1)

Two functions of dendritic cells are believed to be important for the ability of DCs to induce Th1 cells and cytotoxic T-lymphocytes (CTLs), (the types of immune cells most desirable in cancer immunotherapy): high co-stimulatory activity and high production levels of anticancer cytokines, especially IL-12.[31,33-35] Clinical trials to date have relied on the use of either fully matured DCs exhibiting high stimulatory function, but low IL-12 secretion, or immature DCs that display low stimulator/high IL-12 secretion functions. We have recently developed a novel culture method to generate mature DCs that are both highly stimulatory and produce exceedingly high levels of IL-12.[36] Such DCs will be referred to as type-1 polarized DC or αDC1. We have recently succeeded in adapting our original αDC1 protocols based on fetal bovine serum-supplemented cultures to allow αDC1 generation in serum free media, allowing for the application of αDC1s in clinical trials of cancer immunotherapy. Our in vitro observations show that such type-1-polarized DCs (αDC1s) induce up to 50-fold higher frequencies of tumor-specific CTLs during in vitro sensitizations when compared to conventionally matured DCs. Furthermore, these same αDC1s are highly effective inducers of tumor-specific Th1-type $CD4^+$ T cell responses.

Beyond merely exhibiting the unique combination of high immunostimulatory function and high production capacity for cytokines, αDC1s exhibit a stable phenotype that is resistant to PGE2, the suppressive factor over-expressed in colorectal cancer. αDC1s can produce IL-12p70 upon interaction with $CD4^+$ T cells that are unable to produce IFNγ or other IL-12 co-inducing factors.[36,37] This suggests the possibility of using αDC1s to boost the clinical efficacy of cancer vaccines, despite the presumed immunosuppressive environment of immunocompromised (Th2-, Th3- or Tr1-dominated) cancer patients.

αDC1s, the new type of dendritic cell vaccine developed by our group, are the serum-free, clinically-applicable version of type-1 polarized DCs, combining a fully-mature phenotype and high expression of co-stimulatory molecules with an elevated, rather than exhausted ability to produce IL-12p70.[38] In addition to their elevated ability to induce high numbers of IFNγ-producing $CD8^+$ T cells recognizing MHC class-I restricted tumor-related antigens, αDC1s (in contrast to standard mature DCs) have been shown to convert non-cytolytic $CD8^+$ T cells into high perforin- and granzyme B-expressing effector cells with high killer activity and tumor-homing potential.[39] Our clinical trials of αDC1 vaccines used as a single agent in end-stage cutaneus T cell lymphoma (CTCL) and metastatic melanoma showed preliminary evidence of the ability of αDC1s loaded with autologous tumor (CTCL) or antigenic peptides (melanoma) to induce disease stabilizations and clinical responses.[1] However, the most promising data so far results from our recently published Phase 1/2 study in patients with high-grade malignant glioma, showing that the combination of intranodal immunization with αDC1-vaccines with systemic intramuscular (IM) administration of poly-ICLC, capable of enhancing tumor production of CXCR3 ligands (such as CXCL10) in preclinical models, is capable of inducing long-term stabilization of this aggressive disease and objective clinical responses. Within the cohort of 22 patients with recurrent high-grade gliomas (expected progression-free survival of 2-4 months), nine patients achieved the PFS of at least 12 months, with two patients achieving complete radiologic responses and two additional patients undergoing partial (and sustained) responses (2PR and 1CR occurred after the publication of our JCO 2011 report). As expected, the ability of αDC1s from the individual patients to produce IL-12, the functional hallmark of polarized αDC1s and the predictive factor of *in vitro* potency of different types of DC vaccines was the best predictive factor of the ability of αDC1s + poly-ICLC therapy in promoting the progression-free survival of the treated patients.[40]

Interferons, Toll-like receptor (TLR)-ligands, and prostanoid inhibitors, increase the production of effector (T_{eff})-attracting chemokines, and reduce the production of T_{reg}-attracting chemokines. Our recently-published data and data from others, demonstrated that DCs developing in different inflammatory conditions can preferentially attract either effector-type immune cells (CTLs, Th1, and NK cells), desirable in the setting of cancer immunotherapy, or undesirable T_{regs} cells.[4,41] Our data demonstrated the key role of IFNα, poly-I:C (TLR3-ligand), and prostanoid inhibitors (celecoxib), in suppressing the ability of DCs and colorectal cancer tissues to attract T_{regs} and promoting T_{eff} attraction.[4,42] Since αDC1-induced $CD8^+$ T cells express particularly high CTL activity and express the typical T_{eff}-associated chemokine receptors (CCR5 and CXCR5) [39], such chemokine-modulating therapies are likely to be particularly effective when combined with αDC1 vaccination**.** Our latest data from ovarian cancer patients demonstrates that the combination of 3 clinically-applicable factors, IFNα, TLR3 ligand, and celebrex, allows not only a further enhancement of the production of T_{eff}-attracting chemokines, but can also suppress the overproduction of CCL22 a T_{reg}-recruiting chemokine, which has been previously implicated in accelerated progression of ovarian cancer.[43]

2.6 Chemokine Modulatory Regimen

2.6.1 Critical role of chemokine-driven T cell migration in cancers

The numbers of effector T cells in malignant peritoneal mesothelioma and CRC tissues have been shown to be an independent prognostic marker, with high numbers predicting a delayed

time to cancer recurrence.[5-8] In contrast, regulatory T cells in CRC patients are associated with poor tumor response.[44-46] Chemokines and their respective receptors are critical for T cell migration and homing into tissues during homeostasis and inflammation.[47,48] Several studies have shown that CXCR3 and CCR5 are critical for T cell entry into tumors, which is guided by intra-tumoral expression of respective chemokines. High levels of the chemokine ligands for CCR5 (CCL5/RANTES and CXCR3/IP10) in tumor tissue have been associated with infiltration of CD8 T cells with respective receptors in CRC,[49] as well as melanoma[50] and gastric cancer.[51] High $CXCR3^+$ T cells in peripheral circulation has been associated with increased survival of stage III melanoma patients [52]. In contrast, high levels of CCL22 (ligand for CCR4) expression by DC or macrophages in ovarian ascites recruit regulatory T cells and is associated with decreased patient survival[43]. But most tumors do not have T cell infiltration, or express the correct chemokines for effector T cell (T_{eff}) entry. Colon tumors frequently show elevated CCL22 levels induced by a PGE_2 rich tumor microenvironment [53,54] permitting regulatory T cells (T_{reg}) rather than T_{eff} cells, leading to highly biased T_{reg}/T_{eff} ratio in tumors. The current study attempts to correct the above mentioned chemokine bias in the tumor microenvironment using a chemokine modulation regimen and analyze whether such modulation can help $T_{eff,}$ entry and prevent T_{regs} influx into tumors.

2.6.2 Past experience with chemokine modulation agents TLR3 ligands, IFNα, and celecoxib

In our preclinical ex vivo studies performed using explants of resected metastatic CRC, the combination of IFNα with nonselective or COX-2 selective inhibitors of prostaglandin synthesis resulted in strong elevation of production of the effector T cell-attracting chemokines CXCL10 and CCL5 with the concomitant suppression of the intratumoral expression of CCL22, a T_{reg}-attracting chemokine. However, in a subset of patients, the optimal results, particularly with regard to CCL5 induction, required additional stimulation by TLR ligand, poly-I:C. Therefore, we propose to incorporate in this study a combination of the above 3 factors (IFNα; COX-2 selective inhibitor, celecoxib; and a TLR3 ligand, rintatolimod, a short half-life derivative of Poly-I:C).

Interferon–alpha (IFNα, Intron A, Interferon alfa-2b) is currently FDA approved for the adjuvant treatment of stage III melanoma. It has been examined by multiple investigators as an immune modulator in the setting of vaccine therapy with demonstrated safety. It has been extensively evaluated in the setting of metastatic CRC (as a single agent or in combination with chemotherapy or IL-2) and was shown to be largely ineffective at daily doses ranging from 3-20 MU/m^2, administered subcutaneously or intravenously.[55-72] Vaishampayan et al administered IFNα (5 MU subcutaneously three times a week) following vaccination with a melanoma vaccine which was well tolerated (Melacine).[73] Mitchell et al recently completed a large trial examining IFNα (5 MU subcutaneously three times a week) for one year[74]. In a small study by Astsaturov and colleagues, administration of IFNα in previously vaccinated melanoma patients demonstrated objective clinical responses, suggesting its ability to amplify the effectiveness of local T cells infiltration and/or function at the tumor site.[75]

Celecoxib (Celebrex) has been extensively studied in long term clinical trials in patients predisposed to development of colorectal cancer.[76,77] However, there is to our knowledge, no clinical data examining its use as a short term immune modulator in the setting of vaccine therapy. Several studies in murine models have suggested improved efficacy of anti-tumor

vaccination with Cox-2 inhibition.[78-80] Recent literature has suggested increased risk of adverse cardiovascular events associated with the administration of some selective Cox -2 inhibitors.[81-83] The literature regarding the precise risk with celecoxib remains controversial, with the reported cardiovascular side effects observed in its long-term users. This trial will involve a short course of celecoxib; given at an oral dose of 200 mg daily starting on the day of discharge from surgery and continuing until the off-study treatment visit. The dose will be 200 mg, twice a day, the day of vaccination (Monday) and over the 4 days of chemokine modulation regimen (Tuesday-Friday). It has been suggested that lower doses and shorter length of administration decrease the risk of adverse cardiovascular events associated with administration of celecoxib.

Poly-I:C (polyinosinic-polycytidylic acid) is a small molecule ligand (mimicking double-stranded RNA ligand of Toll-Like Receptor 3 [TLR3],[84,85] extensively tested as a single drug in the setting of infectious diseases and cancer. It has been shown that TLR3 is involved in the recognition of viral components, such as poly-I:C and dsRNA. In contrast, TLR7 and TLR8 appear to recognize ssRNA viruses and ssRNA, respectively.[86] It is also noted that IFN-α, β and/or γ treatment of cells results in the remarkable enhancement of TLR3 expression levels, and that poly-I:C is capable of inducing the production of pro-inflammatory cytokines (IL-6 and TNFα) and chemokines (CCL2/MCP-1, CCL5/RANTES, CCL20/MIP-3α, and CXCL10/IP-10). Other TLR ligands, such as LPS, lipoteichoic acid, peptidoglycan, flagellin, and CpG, failed to exert similar effects. These reports, in addition to our preclinical data, provide strong rationale for the combined use of poly-IC and IFNα. With regard to clinical use of poly-I:C in cancer patients, it has been shown that low dose of poly-I:C is more efficacious than a high dose for enhancing immune effects, and that higher doses actually inhibit a number of cell-associated immune functions. Rintatolimod (Ampligen) is a poly-IC derivative with reduced half-life in vivo and increased selectivity of binding to TLR3.

2.6.3 Clinical experience with chemokine modulating regimen (CKM)

In 2012, we completed an Expanded Access - single patient IND (BB-IND 14, 934) protocol utilizing the CKM regimen of IFN intravenously at 5 MU/m^2, rintatolimod intravenously at 200 mg, and celecoxib orally at 200 mg twice a day. This regimen was well tolerated in this patient with grade 3 flushing and hypotension that resolved with slowing of the rintatolimod administration rate on the first cycle.

Similar CKM treatment is currently being evaluated in a Phase 1/2 clinical trial, UPCI 10-131 (IND 112532), open for patients with resectable, metastatic, colon cancer. In that trial, rintatolimod is administered daily and not every second day, as in the current trial. This trial evaluates the CKM regimen of IFN intravenously (dose escalation of 5, 10, and 20 MU/m^2), rintatolimod intravenously at 200 mg, and celecoxib orally at 200 mg twice a day. Treatment on phase I has been completed without any regimen limiting toxicities and current enrollment is to Phase 2 portion of the clinical trial, using the dose of IFNα of 20 MU/M^2.

2.7 Rationale

The current trial tests the safety and clinical effectiveness of the inclusion of personalized patient-specific immunotherapy in the clinical management of peritoneal surface malignancies

(including but not limited to peritoneal carcinomatosis of colorectal-appendiceal and mesothelioma origin), the disease characterized by a uniformly poor overall prognosis and managed with the combination of surgical cytoreduction, local and systemic chemotherapy, similar in all subtypes.

The tested combination immunotherapy will simultaneously target a) each patients' lymphocytes specific to unique tumor-associated antigens overexpressed on each patient tumors, and b) tumor microenvironment (tumor stroma and infiltrating cells) in order to mobilize host's immune responses against their own tumors and to counteract local immune suppression typical of the peritoneal cavity.

We expect to uniformly achieve this goal by:

a) Immunizing each patient with his/her own polarized dendritic cells (αDC1s) loaded with their tumor material as the source of unique patient/tumor-specific antigens (rather than using a pre-determined synthetic antigen), in order to induce the most relevant pattern of immunity in each patient;
b) Utilizing our recently-developed triple CKM regimen which targets peritoneal stromal cells and local inflammatory infiltrate (rather than tumor cells themselves) in order to condition the peritoneal environment and promote selective attraction of the desirable effector cells (CTLs, TH1 and NK cells), while eliminating the local attraction and suppressive functions of the undesirable immune cells (T_{regs} and MDSCs).

We anticipate that such personalized immune therapy will provide a complementary, indirect, and therapeutic synergy with chemotherapy (which directly targets tumor cells), jointly resulting in delayed time to disease progression.

2.7.1 Rationale for intranodal and intradermal administration of αDC1 vaccines

The optimal route of DC administration has not been determined. Our recently completed trial in glioma patients used an intranodal (IN) route of vaccine delivery.[40] In the current trial, we would like to administer a portion of the vaccine via an intradermal (ID) route in order to facilitate additional prolonged stimulation of the immune system by slowly migrating DCs.

2.7.2 Rationale for the combination of αDC1 vaccine and the proposed chemokine-modulating regimen

Based on our preclinical observations that αDC1 vaccine induce high levels of two chemokine receptors, CCR5 and CXCR3 on tumor-specific CTLs[39] and the proposed combination of IFNα, TLR3 ligand and COX2 inhibitor selectively increase the tumor production of the chemokine ligands for these receptors CCR5 ligand: CCL5; and CXCR3 ligands: CXCL9 and CXCL10)[42], we expect that the **proposed combination will be able to promote tumor immune surveillance by a) inducing high numbers of tumor-specific CTLs (αDC1 component of treatment) and b) directing them to the tumor tissues (CKM component),** thus establishing local immune surveillance within the peritoneal cavity and prolonging the time to disease recurrence.

3. PATIENT SELECTION

Consent will be obtained for this study on all eligible patients by the investigator or sub-investigators. No patient will be entered into this clinical trial without having signed a consent form. The research nurse coordinator or designee will screen the patients further, as per investigator/sub-investigator orders, to determine if they meet all the inclusion/exclusion criteria. If a recruited patient is excluded or resigns for any reason during the course of study, a new patient will be enrolled. However, such patients may continue to be followed and/or receive any available vaccine doses.

Candidates for this trial will have peritoneal surface malignancies and CRS + HIPEC is clinically indicated as the first line treatment. Due to the autologous nature of the vaccine, tumor tissue and the cells obtained from the leukapheresis procedure are required. Without those the vaccine cannot be manufactured and the patients are not eligible for this clinical study.

Surgical outcome of must be deemed able to undergo optimal CRS defined as CC-score of 0 or 1 based on post-surgical imaging. Cytoreduction is defined as the burden of residual disease nodules left at the end of surgery (CC-0: no visible disease; CC-1: residual tumor nodules ≤ 2.5 mm in size; CC-2: residual tumor nodules 2.5 mm – 2.5 cm in size; CC-3: residual tumor nodules > 2.5 cm in size).

Screening will occur after recovery from surgery to ensure they meet criteria for treatment.

3.1 Pre-surgery Eligibility

1. Histologically confirmed peritoneal surface malignancies, including but not limited to malignant peritoneal mesothelioma and peritoneal carcinomatosis (PC) from presumed appendiceal and colorectal primary tumors.
2. The following malignancies are ineligible for this study: low grade appendiceal and pseudomyxoma peritonei.
3. Clinically indicated for optimal CRS+HIPEC
4. At least 18 years of age
5. Able to understand and willing to sign a written informed consent document

3.2 Post-surgery Screening

3.2.1 Inclusion Criteria

1. Must have had HIPEC during surgery
2. Must have a CC score of 0
3. ECOG performance status of 0, 1 or 2
4. Able to swallow pills
5. Platelet ≥ 75,000/μL
6. Hemoglobin ≥ 9.0 g/dL
7. Hematocrit ≥ 27.0%
8. WBC >2000/mm^3
9. Creatinine < 1.5 x institutional upper limit of normal (ULN), OR Creatinine clearance ≥ 50

mL/min/1.73 m^2 for patients with creatinine levels greater than 1.5 x ULN
10. Total bilirubin ≤ 1.5 x ULN
11. AST(SGOT) and ALT(SGPT) ≤ 2.5 X ULN
12. Must be eligible for pheresis within 8 weeks of surgery
13. Availability of sufficient number of tumor cells for cryopreservation and subsequent vaccine production

3.2.2 Exclusion Criteria

1. Infection of tumor tissue with pathogens resistant to radiation and fungizone
2. Patients on systemic immunosuppressive agents, including steroids. Patients who are able to be removed from immunosuppressives at least 5 days prior to the first vaccine will be considered eligible
3. Patients with active autoimmune disease or history of transplantation. Patients with indolent or chronic autoimmune disease not requiring steroid treatment are considered eligible.
4. Patients who are pregnant or nursing
5. Patients experiencing a cardiac events (acute coronary syndrome, myocardial infarction, or ischemia) within the 3 months prior to accrual
6. Patients with a New York Heart Association classification of III or IV
7. Prior allergic reaction or hypersensitivity to celecoxib or NSAIDs

3.3 Participation of children

Children under the age of 18 years will not be considered as potential candidates because the incidence of peritoneal surface malignancies in the pediatric age group is extremely low and the fact that we do not have data to show that the proposed experimental treatment is feasible and safe to give to children under the age 18 years of age. If new information becomes available to show the proposed treatment has minimal risks when compared to the benefits and is feasible in pediatric patients, the protocol will be amended to include them.

3.4 Women and pregnancy

Because the effect of the proposed investigational treatment was not studied in pregnant women the potential risks to the fetus cannot be assessed, pregnant females will not be included in the study. However, no female of childbearing potential will be excluded from the study. An effective form of contraception of the woman's choice will be required at all times during study. A pregnancy test will be performed on enrolled women of childbearing potential. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.5 Minority enrollment

The racial and ethnic characteristics of the proposed patient population will reflect the demographics of Pittsburgh and the surrounding area and/or the patient population of the UPMC Health Systems. We shall attempt to recruit patients in respective proportions to these demographics. No exclusion criteria will be based on race, ethnicity, gender, or socioeconomic status. Patients with immune deficiencies such as human immunodeficiency virus infection will

be excluded. The numbers of enrolled male to female patients will be consistent with and reflective of the numbers of patients seen in the UPMC clinics and in approximate 50:50 proportions. No minorities or their subpopulations will be excluded from the study. Every attempt will be made to encourage participation of minorities in these trials.

4. REGISTRATION PROCEDURES

N/A (Single-Institution Study)

5. TREATMENT PLAN

Prior to starting study treatment patients will undergo CRS + HIPEC surgery. A portion of the resected tumor, removed as part of the patient's clinical care for their disease, will be evaluated for the number of cancer cells as a part of eligibility screening procedures. This tissue will be used to manufacture each patient's autologous vaccine (autologous tumor loaded on the patients' autologous dendritic cells). The beginning of postoperative immunotherapy (defined as week 1) will be based upon recovery from CRS + HIPEC, but no sooner than 6 weeks post-surgery, at the discretion of the investigator/sub-investigator, to allow for adequate recovery from surgery.

Immunotherapy are intended to be administered in the UPCI-CTRC. However, based upon scheduling and logistics those procedures are not limited to UPCI-CTRC and may be administered in equivalent locations at UPMC Shadyside and/or the Hillman Cancer Center.

5.1 Phase 1: Dose assessment of αDC1 vaccine chemokine-modulatory regimen (CKM)

The recommended Phase 2 dose of the combination of αDC1 vaccine in combination with IFNα, rintatolimod, and celecoxib will be established by administering fixed doses of the vaccine, rintatolimod, and celecoxib in combination with escalating doses of IFNα. Patients will receive a priming dose of the vaccine which consists of 1 intranodal injection followed by 1 intradermal injection on the Monday of the first week. Patients will then receive a booster dose of the DC vaccine, which consists of 1 intranodal injection and 1 intradermal injection, on Monday followed by the CKM regimen on days Tuesday through Friday of the booster cycle.

There will be 3 dose finding cohorts with one additional de-escalation cohort (designated -1) if needed, as shown in the table below:

Phase 1 Dose Tiers	**αDC1 Vaccine (IN + ID)*****	**Components of CKM cycle**		
		IFNα (MU/M^2 IV) Days T-F	**Rintatolimod* (mg, IV) Days W&F**	**Celecoxib** (mg, orally, twice a day) Days T-F**
-1	3+3 M cells	5	**100**	200
1	3+3 M cells	**5**	200	200
2	3+3 M cells	**10**	200	200
3	3+3 M cells	**20**	200	200

*Rintatolimod starting dose will be 200 mg unless dose escalation is from tier -1 in which case cohorts 2 and 3 will use 100 mg of rintatolimod.
**Celecoxib will be administered orally, at a dose of 200 mg, once a day starting the day of the first vaccine (day 1 of week 1) and continue until Friday the last day of the CKM regimen. The dose will be increased to 200 mg, twice a day on the days of vaccination (Monday) and over the 4 days of chemokine modulation regimen (Tuesday - Friday).
***In case of limited availability of vaccine, the treatment may proceed with reduced numbers of the cells (down to 0.5 M IN + 0.5 M ID for each vaccination cycle).

Dose escalation will start with dose tier 1 and proceed to dose tier 2 in the absence of RLTs. Otherwise the dose will be de-escalated to tier -1. The escalation/de-escalation schema uses the following table. Under this schema, more than one patient can be enrolled at the same time; however, ***within Phase 1, CKM cannot be started on the next patient until the previous patient completes the cycle of CKM and an additional one week of observation for potential RLTs (total of two week difference between the consecutive patients). Also, CKM cannot be started on the next dose until 2 patients on the lower dose complete the CKM regimen and additional one week of observation for potential RLTs.*** Similar rule will be applied during the escalation between different tiers and for the patients within the same dose tier, in order to allow time to evaluate any potential toxicities.

The establishment of the RP2D will be based on an adaptive design for which the next dose will be set according the following rules:

Dose Escalation/De-escalation Rules

Observed Number of RLTs on a Dose Tier	Decision Rule for the Next Subject
There is at least 1 RLT among the last 2 subjects treated on this dose tier and the proportion of DLTs on this dose tier is > 0.30	Assign to next lower dose
There are no RLTs among the last 2 subjects treated on this dose tier and the proportion of RLTs among all subjects on this dose tier is ≤ 0.30	Assign to next higher dose
Neither of the above is satisfied	Assign to the same dose

If no RLTs are observed among the first 3 dose tiers, four additional patients will be added to tier 3 for a total of 6 on tier 3. If, after treating 10 patients without RLT (2 + 2 + 6), dose tier 3 will be declared the RP2D. If an RLT is observed at any dose tier the adaptive up-and-down design will commence until 15 patients are observed after which the Phase 2 starting dose will be estimated by isotonic regression.

Regimen limiting toxicities (RLTs) are defined as the following toxicities:

Vaccine possibly, probably or definitely related RLTs
- Grade 2 or more bronchospasm or generalized uticaria (hypersensitivity)
- Grade 2 or more allergic reaction
- Grade 3 injection site reaction due to vaccine
- > Grade 3 toxicity of any kind related to the DC vaccine

CKM possibly, probably or definitely related RLTs

- Grade ≥ 3 leukopenia or neutropenia persisting ≥ 4 days (96 hours) after treatment
- Greater than or equal to grade 3 non-hematologic adverse events, except for the following grade 3 toxicities unless they last greater than one week:
    - Flu-like symptoms/constitutional symptoms (i.e., fever, chills, arthralgias, myalgias)
    - Depression, anxiety, restlessness, or insomnia
    - Manageable GI symptoms (i.e., nausea, diarrhea)

The observation period for each patient in the Phase 1 portion of this study will be up to 5 weeks (i.e. up to and including the Friday 1 week after the end of the chemokine modulating regimen).

Note: The last 2 patients in the Phase 1 cohort that determines the RP2D will continue on to the Phase 2 part of the trial and will receive the 2nd and 3rd booster/CKM cycles and follow-up per the Phase 2 portion of this protocol. All patients in the dose finding cohort will be followed for primary efficacy endpoints as well as have blood and serum drawn for corollary studies.

Patients in the Phase 1 (safety) cohort will receive their study treatment and will be monitored for RLTs according to the following timeline:

Eligibility	ECOG performance status, blood work
CRS + HIPEC	
Screening	History and physical exam, ECOG performance status, blood work, and evaluation of tumor material
Post-surgery (after the discharge from the hospital)	Leukapheresis
Week 1* (upon recovery from surgery; at least 4 weeks after surgery)	**Priming Vaccine dose; no chemokine modulation** Monday: αDC1 vaccine Oral celecoxib, 200 mg, twice daily on the day of vaccination Tues – Sun: oral celecoxib 200mg, once daily
Weeks 2-3	Rest – continue oral celecoxib, 200 mg, once daily
Week 4	**Cycle 1 ("booster cycle")** Monday: αDC1 vaccine Tues – Fri: Systemic Chemokine Modulation Regimen. Oral celecoxib, 200 mg, twice daily on the days of vaccination and CKM. Celecoxib will be discontinued after CKM on Friday. Rintatolimod is only administered on Wednesday and Friday.
Off study treatment	Follow-up for Time-to-Progression/occurrence (TTP)

CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemoperfusion; TTP: Time-to-progression
*Adjuvant immunotherapy may be delayed until full recovery at the discretion of the investigator/sub-investigator. The beginning of immunotherapy will be considered week 1 of the experimental treatment and the timing of all subsequent elements of treatment will be based upon the first vaccine.

5.2 Phase 2: Efficacy assessment of αDC1 vaccine followed by the chemokine-modulatory regimen (CKM)

Study treatment will consist of one priming dose of DC vaccination followed by 3 booster cycles of DC vaccine + chemokine modulation regimen (CKM), cycles 1-3. The booster vaccine is administered on Monday followed by 4 days of the CKM regimen (Tuesday – Friday).

Timing of the experimental treatment:

- Priming cycle: 6 to 16 weeks after surgery (target: 8 weeks)
- Booster cycle 1: 4 to 12 weeks after priming cycle (target: 4 weeks)
- Booster cycle 2: 4 to 12 weeks after booster cycle 1 (target: 4 weeks)
- Booster cycle 3: 4 to 12 weeks after booster cycle 2 (target: 4 weeks)

It is expected that a significant proportion of the patients will undergo adjuvant clinically indicated chemotherapy. Adjuvant chemotherapy can be administered between the courses of immunotherapy, but no sooner than 5 days after finishing any cycle of immunotherapy. The following cycle of immunotherapy will start no sooner than 5 days after the last day of a chemotherapy cycle and after recovery from acute side effect of chemotherapy.

Patients who (for clinical or logistic reasons) will not be able to take any cycle of immunotherapy within the above-indicated windows, can still receive the remaining courses of immunotherapy at later time points, but will not be included in the analysis of the primary efficacy endpoint, although they can be analyzed in an exploratory manner.

Similarly, patients who (for clinical or logistic reasons) will not be able to receive the priming dose of vaccine within 3 months after surgery, can still receive immunotherapy at later time points, but will not be included in the analysis of the primary efficacy endpoint, although they can be analyzed in an exploratory manner.

In the efficacy phase, patients will receive a priming dose of the αDC1 vaccine followed by 3 courses of the αDC1 booster vaccine combined with CKM regimen as illustrated in the following table:

Eligibility	Informed Consent
CRS + HIPEC	
Screening	History and physical exam, ECOG performance status, blood work, evaluation of tumor material
Post-surgery (within the first 8 weeks following surgery)	Leukapheresis
Week 1* (upon recovery from surgery; no sooner than 6 weeks post-surgery)	**Priming Vaccine dose; no chemokine modulation)** One day: αDC1 vaccine Oral celecoxib, 200 mg, before and after treatment on the day of vaccination
Weeks 2-3	Rest
Week 4 (target)*	**Booster Cycle 1** Monday: αDC1 vaccine

	Tues – Fri: Systemic Chemokine Modulation Regimen. Oral celecoxib, 200 mg, twice daily on the days of vaccination and CKM. Celecoxib will be discontinued after CKM on Friday. Rintatolimod is only administered on Wednesday and Friday.
Week 5-7	Rest
Week 8 (target)**	**Booster Cycle 2** Monday: αDC1 vaccine. Reintroduce oral celecoxib, 200 mg, twice daily on the days of vaccination and CKM. Tues – Fri: Systemic Chemokine Modulation Regimen. Rintatolimod is only administered on Wednesday and Friday. Celecoxib will be discontinued after CKM on Friday.
Week 9-11	Rest
Week 12 (target)**	**Booster Cycle 3** Monday: αDC1 vaccine. Tues – Fri: Systemic Chemokine Modulation Regimen. Rintatolimod is only administered on Wednesday and Friday. Oral celecoxib, 200 mg, twice daily, will be given on the days of vaccination and CKM. Celecoxib will be discontinued after CKM on Friday.
Off study treatment	Follow-up for Time-to-Progression/occurrence (TTP)

CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemoperfusion; TTP: Time-to-progression

*Adjuvant immunotherapy may be delayed until recovery at the discretion of the investigator/sub-investigator. The beginning of immunotherapy will be considered week 1 of the experimental treatment and the timing of all subsequent elements of treatment will be based upon the first vaccine.

**Patients may receive postoperative (standard of care, adjuvant) chemotherapy at the discretion of the treating oncologist. Adjuvant chemotherapy may start 5 days after the first full cycle of immunotherapy (first booster vaccine + CKM). Following completion of adjuvant chemotherapy, subsequent immunotherapy can start after 5 days post adjuvant chemotherapy.

5.3 Leukapheresis

Prior to the procedure each patient's venous access will be evaluated. If a patient does not have acceptable venous access a pheresis catheter will need to be inserted into a central vein by qualified personnel and removed directly after leukapheresis. A separate consent form for central venous access will be obtained for such a scenario.

All selected patients will undergo leukaphersis after CRS + HIPEC surgery and screening but before the priming dose of vaccine. The product is delivered immediately to the University of Pittsburgh Cancer Institute Immunotransplantation Center (ITC). The leukapheresis product will be immediately processed as described in the Chemistry, Manufacturing, and Control section of the BB-IND affiliated with this protocol and will be cryopreserved as described.

5.4 Agent Administration

5.4.1 Celecoxib

The drug will be administered orally, at a dose of 200 mg, twice daily on all study treatment days only.

5.4.2 DC vaccine administration

The intranodal and intradermal administration of the DC vaccine will be performed by the investigator/sub-investigator or their designee.

5.4.2.1 *Intra-nodal administration*

The priming dose of vaccine may be administered on any day of the week since it is not followed by the CKM regime. The intranodal injection for the booster doses will be administered on Monday of cycles 1 through 3.

Intranodal administration will consist of ultrasound-guided intranodal injections of the autologous tumor αDC1 vaccine at a target dose of 3×10^6 in 0.5 mL. Due to manufacturing limitations a minimum of 0.5×10^6 cells is acceptable. In order to avoid repetitive scarification of a single lymph node, the patients will have subsequent doses administered at 3 different sites, with the sites rotated as follows: (a) right inguinal lymph nodal group, (b) left inguinal lymph nodal group, (c) right axillary lymph nodal group, and (d) left axillary lymph nodal group. Only one lymph node will be injected at each visit with the dendritic cells. The size of the lymph node to be injected will be a minimum of 5 mm and a maximum size of 20 mm.

5.4.2.2 *Intradermal route*

Intradermal injection will be used to complement the intranodal injections (provide a depot effect) and it can be used as the only route (for all DCs) in case intranodal injection is not feasible. Each portion of the vaccine will be administered using a 1 mL syringe and needle appropriate for intradermal injections and will hold 0.5 mL of the vaccine preparation. The intradermal DC vaccine should be injected in the vicinity of the major nodal basin of the thigh. The nodal basin must not have been previously dissected.

5.4.3 Tumor-selective systemic chemokine modulation regimen (CKM)

The chemokine modulating regimen will be administered over 4 consecutive days (Tuesday to Friday) in the following order:

- Pretreatment: 500 mL Normal saline IV over approximately 60 minutes
- Pre-meds: Acetaminophen (Tylenol) 650 mg x 1 dose; Prochlorperazine (Compazine) 10 mg x 1 dose – administered 30 minutes (±5 minutes) after starting pre-treatment hydration
- Celecoxib: 200 mg orally, administered any time prior to IFN
- IFN: IV over 20 minutes (±5 minutes)
- Celecoxib: 200 mg orally followed at least 15 minutes later by rintatolimod
- Rintatolimod: 200 mg (or 100 mg in case of de-escalation) IV, Wednesday and Friday only

of the CKM regimen, initial administration should begin at a slow rate of infusion (approx. 20 cc/hour) and increase to 40cc/hour after approximately 30 minutes. Tubing should be flushed with 30 to 50 mL of normal saline solution upon completion, Administration will be followed by 1 hour of observation and vital signs at 30 (±5 minutes) and 60 minutes (±10 minutes)

Pretreatment and pre-meds listed above are recommendations and may be modified based upon institutional SOC requirements and at the discretion of the investigator/sub-investigator.

5.5 Continuation of Booster Vaccines Administration

At the discretion of the treating physician, any patient who completes study treatment or is removed for reasons other than vaccine related AE/SAEs may continue to receive available vaccine. These vaccines may be administered 4-12 weeks apart. Subjects removed will continue to be evaluated for safety, but not be included in the analysis of formal efficacy objectives but may be evaluated in an exploratory manner. These subjects will be evaluated/followed based upon the conventional care evaluations outlined in the study calendar on days of vaccination (i.e., PE, vitals, AE evaluation).

5.6 Safety evaluation and monitoring

The recommended starting dose of the combination of vaccine and chemokine modulation will be determined in the Phase 1 portion of the trial. In order to refine the estimate of the RLT rate during the efficacy phase we will apply continual Bayesian monitoring of treatment-related regimen limiting toxicities and, if needed, impose a stopping rule that permits suspension of the trial by the investigator for review by the Data Safety and Monitoring Committee (DSMC). Details and a monitoring table are in section 13.

5.7 Duration of Study Treatment

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression (excluding disease progression during the time between surgery and the CKM regimen, these patients will be allow to continue with study treatment). See also Section 5.5.
- Patient withdraws consent but allows continued follow-up for survival
- Patient non-compliance
- Unacceptable agent specific adverse event(s).
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator/sub-investigator.
- Pregnancy in patient

The reason and date a patient was removed from study treatment must be documented.

5.8 Off Study Treatment Visit

Within 1-2 weeks after the last day of study treatment, patients will have their last study specific clinical visit. At the discretion of the treating physician, the tests/procedures due to be performed at the Off Study Treatment visit may be performed on the last day of the CKM treatment for scheduling purposes.

AEs and conmeds will be collected for up to 5 days post last dose of treatment. Any patient experiencing study related SAE will be followed until resolution to Grade 1 or baseline.

5.9 Duration of Follow Up

Patients will be followed until death and the follow-up information for trial reporting will be obtained either from the patient's medical records or through direct contact with the patient (i.e. clinic visit or phone call). The timing of these will be based on scheduling of clinical care for the treatment of the patient's cancer (i.e. clinic visits every 3 months, 6 months, yearly). Patients will be followed specifically for evidence of progression/recurrence based on imaging and survival.

5.10 Duration of Study

Patients will be removed from study if any of the criteria below applies.

- Patient decides to withdraw from the study.
- Patient removed due to non-compliance.
- The investigator decides to prematurely close the trial.

The reason for study removal and the date the patient was removed must be documented.

6. DOSING DELAYS/DOSE MODIFICATIONS

Each vaccination and/or chemokine-modulation regimen may be delayed for up to 8 weeks and all subsequent cycles will be adjusted accordingly.

6.1 Phase 1

Any patient who experiences a RLT will be removed from study treatment and followed until the RLT resolves to Grade 1 or baseline.

6.2 Phase 2

After cycle 1, any AE experienced by a patient must resolve to Grade 1 or baseline prior to receiving adjuvant chemotherapy.

6.2.1 DC vaccine

Any patient who experiences the following, and is attributable to the DC vaccine, will be removed from study treatment:

- Grade 2 or more bronchospasm or generalized uticaria (hypersensitivity)
- Grade 2 or more allergic reaction
- Grade 3 injection site reaction due to vaccine
- > Grade 3 toxicity of any kind related to the DC vaccine

6.2.2 CKM

For grade 3 toxicities:

1st episode: dose will be held (until the toxicity is reduced to ≤ grade 1 or baseline) and restarted at the next study visit at a dose reduction of 33% (dose reduction for IFN and rintatolimod, celecoxib will not be dose reduced). The dose can be re-escalated at the next study cycle.

2nd episode: dose will be held and restarted (if toxicity is resolved to ≤ grade 1 or baseline) at the next study visit at a dose reduction of 33%. The dose cannot be re-escalated.

For grade 4 toxicities:

1st episode: dose will be held (until the toxicity is reduced to ≤ grade 1 or baseline) and restarted at the next study visit at a dose reduction of 33% (dose reduction for IFN and rintatolimod, celecoxib will not be dose reduced). The dose can be re-escalated at the next study cycle.

2nd episode: Eliminate the CKM regimen

Patients who are discontinued from the CKM treatment may still receive the DC vaccine.

6.3 Gastrointestinal Toxicity

Nausea and/or vomiting should be controlled with adequate anti-emetic therapy. Prophylactic anti-emetic therapy can be used at the discretion of the treating physician. Patients are encouraged to take plenty of oral fluids. If symptoms persist despite maximal anti-emetic therapy, protocol drugs should be withheld until recovery to ≤ grade 1.

Diarrhea should be managed with appropriate anti-diarrheal therapy. Patients should be encouraged to take plenty of oral fluids. If symptoms do not decrease to grade 1 or less with adequate anti-diarrheal therapy, all protocol drugs should be held until resolved to ≤ grade 1.

6.4 Hypersensitivity Reactions

Caution: patients who had a mild to moderate hypersensitivity reaction have been successfully re-challenged, but careful attention to prophylaxis and bedside monitoring of vital signs is recommended.

Hypersensitivity reactions to IFNα and/or TLR3 ligand will be managed as follows:

Mild symptoms (e.g., mild flushing, rash, pruritus) - complete infusion, supervise at bedside, no treatment required.

Moderate symptoms (e.g., moderate rash, flushing, mild dyspnea, chest discomfort) - stop infusion, give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg, resume the infusion at a low rate after recovery of symptoms, then, if no further symptoms, at full dose rate until infusion is complete. If symptoms recur, stop the infusion and the patient should receive no additional INFα or TLR3 ligand for that cycle, but may be retreated after discussion with the Investigator. Keep a record of the toxicity.

Severe life threatening symptoms (e.g., hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria) - stop infusion, give intravenous diphenhydramine and dexamethasone as above, add epinephrine or bronchodialtors if indicated. If wheezing is present, that is not responsive to bronchodilators, epinephrine is recommended. Patient should be removed from further protocol therapy. Report it as an adverse event.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

Note that Phase 2 of this study will include two periods of immunotherapy, administered before and after adjuvant chemotherapy (minimum 5 day wash-out periods), in order to separate potential toxicities of the chemokine modulatory regimen (all three factors are short-acting), from the anticipated toxicities of adjuvant chemotherapy. For this reason, we will only collect the toxicity data from the periods of immunotherapy. All toxicities related to the vaccine or CKM must be followed until resolution to grade 1 or baseline.

7.1 Adverse Events and Risks List(s)

7.1.1 DC Vaccine

Dendritic cell vaccines have been investigated extensively in a number of centers and to date, have not been reported to be associated with limiting toxicities or adverse side effects. DC vaccines carry a potential risk of development of autoimmune disease (such as lupus, or vitiligo). Whereas development of vitiligo has been reported with similar vaccines administered to melanoma patients (and correlated with positive response to vaccination), other side effects have not been reported. Although, based on previous trials with DC-based vaccines, the possibility of developing an allergic reaction to DC-based vaccines is rare there is a chance that patients could have an allergic reaction to the vaccine.

While other classes of immunotherapueutic drugs, particularly the currently approved immune checkpoint blocker, Yervoy [Ipilimumab] can produce significant toxicity, such as enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy, due to its ability to promote polyclonal T-cell activation and proliferation, such effects have not been described following application of DC cancer vaccines, including previously-administered alpha-DC1-based vaccines, their combinations with a long acting TLR3 ligand poly-ICLC (Okada,

Kalinski, Bartlett, J. Clin. Onc. 29 (3):330-336, 2011) or their combinations with the proposed CKM regimen in our patients treated on compassionate-use protocols.

7.1.2 Celecoxib

Long term use of celecoxib may cause dyspepsia, headaches (including migraines) and borderline elevated liver function tests (which could indicate liver damage). Recently, information from three long-term studies of celecoxib has become available. In the first study, a cancer prevention study, an increased risk of heart attacks, strokes, and/or deaths resulting from heart or blood vessel disease was reported among people taking celecoxib. Approximately 1 in 100 patients enrolled in this study receiving the placebo treatment had one of these serious events. In contrast, between 2 and 3 in 100 patients taking celecoxib (between 400 and 800 mg daily) had one of these serious events. Another clinical cancer prevention study found no increased risks in patients taking celecoxib 400 mg daily. The third study, an Alzheimer's disease prevention study, did not find increased risks with celecoxib. Dosing has been suspended in all three of these studies based on the findings of the first cancer prevention study. As a result, the FDA is now evaluating the possibility that celecoxib increases the risk of heart attack, stroke, and or death resulting from heart or blood vessel disease. Known infrequent side effects of celecoxib include nausea and/or vomiting, diarrhea, flatulence, abdominal and stomach pain, bleeding ulcer, upper respiratory tract infection, pharyngitis, rhinitis, sinusitis, peripheral edema, back pain, dizziness, insomnia, and skin rash. Rare risks include sudden death (unexpected or instant death that occurs within minutes or hours from any cause other than violence), vasculitis, hepatitis, liver failure, kidney failure, blood dyscrasia, hypoglycemia, hyponatremia, viral meningitis, severe allergic reaction, visual changes, and transient ischemic attack.

Warnings/Precautions: Stomach problems may be more likely to occur if patients drink alcoholic beverages while taking this medicine. Taking two or more of the nonsteroidal anti-inflammatory drugs together on a regular basis may increase the chance of unwanted effects. Also, taking acetaminophen, aspirin or other salicylates, or ketorolac (e.g., Toradol) regularly while taking a nonsteroidal anti-inflammatory drug may increase the chance of unwanted effects. The risk will depend on how much of each medicine is taken every day and how long the medicines are taken together. Therefore, patients should not take acetaminophen or aspirin or other salicylates or ketorolac (e.g., Toradol). Clinical studies with celecoxib have identified potentially significant interactions with fluconazole and lithium. Experience with nonsteroidal anti-inflammatory drugs (NSAIDs) suggests the potential for interactions with furosemide and ACE inhibitors. Celecoxib is contraindicated in patients with known hypersensitivity to celecoxib. Celecoxib should not be given to patients who have demonstrated allergic-type reactions to sulfonamides. Celecoxib should not be given to patients who have experienced asthma, urticaria, or allergic-type reactions after taking aspirin or other NSAIDs. Severe, rarely fatal, anaphylactic-like reactions to NSAIDs have been reported in such patients.

For more risk information reference the Investigator’s Brochure or package insert.

7.1.3 Interferon-α2b

IFNα may cause fever, chills and flu-like symptoms; loss of appetite; nausea; vomiting, diarrhea and abdominal pain; fatigue; lowered white blood count may increase risk of infection; lowered

platelets may lead to an increase in bruising or bleeding; hair loss. Other risks which may be common in cases of prolonged administration include drowsiness; temporary confusion; anxiety, amnesia, irritability, confusion, delusions and depression which can be severe; numbness and/or tingling in the hands and/or feet, skin rashes and inflammation of the pancreas. Inflammation of the pancreas is swelling or irritation of the pancreas which may result in tenderness or pain in the stomach and/or back. When the pancreas is inflamed, the body is not able to absorb all the nutrients it needs.

For more risk information reference the Investigator's Brochure or package insert.

7.1.4 Rintatolimod (poly IC analog)

Clinical experience with rintatolimod totals over 800 patients with more than 400 patients receiving Ampligen® for at least six (6) months, greater than 200 patients for one (1) year, over 50 patients up to two (2) years, and with 20 or more patients over two (2) years at doses as high as 1200 mg i.v. twice weekly. No evidence of dose-limiting organ toxicity, including hematologic, liver, or renal toxicity, has been observed.

Adverse events related to infusion such as mild flu-like symptoms, transient headache, fever, myalgia, arthralgia, and fatigue/malaise which were seen, usually occur during the initial weeks of treatment and tend to subside on repeated administration. These side events were seen in Chronic Fatigue Syndrome patients, cancer patients, chronic hepatitis B infected patients and individuals infected with HIV at doses of 200 and 400 mg and higher. Patients that experience these minor side effects can continue on Ampligen® and as noted, these signs and sympotms typically subside after several weeks of continued treatment. Specific symptoms of note include a flushing reaction, characterized by at least one occurrence of erythema of the face, neck and chest, which has been observed in approximately 10% of patients treated in various studies. Usually the flushing is both mild and transient and disappears with repeated dosing. Occasionally, it can be accompanied by a tightness of the chest, tachycardia, anxiety, shortness of breath, subjective reports of "feeling hot", diaphoresis and nausea. The reaction is usually infusion-rate dependent and may generally be controlled by slowing the infusion rate. An antihistamine (diphenhydramine hydrochloride) can be helpful in controlling and reducing the response in the occasional patient for whom the symptom persists. Other less frequently occurring adverse effects include nausea, diarrhea, itching, urticaria, bronchospasm, transient hypotension, photophobia, rash, bradycardia and transient visual disturbances. A severe unexpected local reaction to extravasation of rintatolimod (Ampligen®, Poly I:Poly $C_{12}U$) at the infusion site in the dorsum of the left hand was reported in a Chronic Fatigue Syndrome patient with chilblains. Several patients experienced liver enzyme level elevations while receiving Ampligen® associated with chronic dosing over many weeks.

Rintatolimod has been dosed in combination with alpha interferon in investigator initiated studies under investigator IND applications during the period between December 1985 and April 1994. A total of 24 patients received combination treatments. Clinical conditions included renal cell carcinoma, chronic myelogenous leukemia, melanoma, and ovarian cancer. Rintatolimod was given as an IV infusion at a dose of 300 mg BIW. The starting dose was sometimes as low as 1-10 mg. The interferons were administered at a dose of 3 million Units daily, with some doses of 0.75 mU at the low side and up to 6 mU at the higher side.

The therapy with rintatolimod in combination with alpha interferon was generally well tolerated without evidence of dose-limiting or cumulative toxicities. The most frequent adverse reactions were considered minor in severity and duration. Most of them were flu-like symptoms such as chills, cold feeling, fatigue, decreased appetite, fever, muscular aches. Also shortness of breath has been seen as well as hypotension, nausea, anemia, dyspnea, numbness, itching and blurred vision. These adverse events were judged possibly related to the condition of the patients, but also possibly related to the administration of rintatolimod or interferon. In some cases worsening of the patient's condition has been seen due to tumor progression, but in general favorable clinical patterns were observed in these patients with advanced disease. Such combinations were not intended to be immune modulating and were evaluated in the context of clinical cancer care.

For more risk information reference the Investigator's Brochure.

7.2 Definitions

Adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Adverse reaction means any adverse event caused by a drug.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction".

> *Reasonable possibility*, for the purpose of IND safety reporting, means there is evidence to suggest a causal relationship between the drug and the adverse event.

Life-threatening, suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator (i.e., study site principal investigator) or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include a suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious, suspected adverse reaction is considered "serious" if, in the view of either the investigator (i.e., study site principal investigator) or sponsor, it results in any of the following outcomes: death, a life-threatening adverse reaction, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

> Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected, suspected adverse reaction is considered "unexpected" if it is not listed in the general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

7.3 Recording/Reporting requirements

7.3.1 Eliciting AE Information

Research subjects will be routinely questioned about AEs at study visits.

7.3.2 Recording Requirements

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s).

AEs or abnormal test findings felt to be associated with the investigational drug or study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator (i.e., study site principal investigator) or sponsor.

7.3.3 Abnormal Test Findings

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy.

  Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical study.
- The test finding is considered an AE by the investigator (i.e., study site principal investigator) or sponsor of the IND application.

7.3.4 Causality and severity assessment

The investigator (i.e., study site principal investigator) or sponsor of the IND application will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable

possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the investigator (i.e., study site principal investigator) or sponsor's final determination of causality is "unknown and of questionable relationship to the study drug(s)", the adverse event will be classified as *associated with the use of the study drug(s)* for reporting purposes. If the investigator (i.e., study site principal investigator) or sponsor's final determination of causality is "unknown but not related to the study drug(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

7.4 Reporting of Suspected Adverse Reactions

All events meeting the definition of a serious adverse event should be recorded on a MedWatch 3500A Form (http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf) or departmental SAE form. Copies should be sent to the Sponsor, Investigator, crssafetysubmissions@upmc.edu, and the local Institutional Review Board per institutional reporting requirements.

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Event Description (section 5) of the MedWatch 3500A form:

- CTCAE term(s) and grade(s)
- current status of study drug
- all interventions to address the AE (testing and result, treatment and response)
- hospitalization and/or discharge dates
- event relationship to study drug

Follow-up reports:
Additional information may be added to a previously submitted report by adding to the original MedWatch 3500A report and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form.

7.5 Review of Safety Information: Sponsor Responsibilities[1]

The sponsor must promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States.

[1] 21 CFR Sec. 312.50

7.6 Review of Safety Information: Investigator Responsibilities[2]

An investigator shall promptly report to the sponsor of the IND application any adverse effect that may reasonably be regarded as caused by, or probably caused by, the drug. If the adverse effect is alarming, the investigator shall report the adverse effect immediately. An investigator shall provide the sponsor with an adequate report shortly after completion of the investigator's participation in the investigation.

7.7 Reporting of Adverse Reactions to the FDA

Reporting of serious adverse events to the FDA will follow 21 CFR 312.32.

7.7.1 Written IND safety reports

The Sponsor will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

7.7.2 Telephoned IND safety reports – fatal or life-threatening suspected adverse reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon

[2] 21 CFR Sec. 312.64

as possible but in no event later than 7 calendar days after the Sponsor's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

7.8 Reporting Adverse Events to the Responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the Investigator's receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and commercial agents administered in this study can be found in Section 7.1.

This Phase 1/2 trial will evaluate the effectiveness of autologous αDC1 (alpha dendritic cell type 1 loaded with autologous tumor material) vaccines, combined with a systemic chemokine modulation regimen [CKM; intravenous rintatolimod (TLR3 ligand, a derivative of Poly-I:C) + intravenous IFNα + oral celecoxib] as adjuvant therapy, after cytoreductive surgery (CRS) and locoregional hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with potentially resectable peritoneal surface malignancies (PSM), including but not limited to malignant peritoneal mesothelioma and peritoneal carcinomatosis (PC) of appendiceal and colorectal origin. The primary outcome measure will be recurrence free survival.

8.1 DC Vaccine

Dendritic cells (DC) are derived from autologous (the patient's own) adherent mononuclear cells (monocytes) in the peripheral blood by 5-day or 6- day culture in the presence of interleukin 4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF), followed by 1-2 days of maturation. The autologous tumor tissue needed for manufacturing of the vaccine is acquired from surgery that is performed as part of the patient's standard of care for their cancer as determined by their treating physician.

8.1.1 Other names

Alpha-type-1 polarized dendritic cells, alpha DC1, αDC1

8.1.2 Formulation

The DC Vaccine will be prepared in the cGMP facility of the University of Pittsburgh Cancer Institute, in a process analogous to the existing BB-IND 13,234 (colorectal cancer). The αDC1 vaccines are prepared in the University of Pittsburgh Immunotransplantation Center (ITC) under cGMP conditions and the final product is vialed and cryopreserved. Labels placed on all tubes contain a unique vaccine lot. The vaccine release will follow the quality and safety testing performed by the ITC. The target dose of αDC1 vaccine is 3×10^6 cells in 0.5 mL for each injection, intranodal and intradermal, however, in case of manufacturing limitations; a minimum of 0.5×10^6 cells is acceptable per injection site (0.5×10^6 cells total). Patients can receive a lesser number of vaccine DCs, but any such patients will not be included in the analysis of the primary endpoint.

8.1.3 Storage and preparation

DCs used in the vaccine will be suspended in 5% human serum albumin (HSA) and delivered to the clinic for administration. For preparation of the vaccines, the labeled vials of cryopreserved αDC1 are removed from storage in liquid nitrogen and quickly thawed in a 37°C water bath. After 3 washes in sterile medium, thawed αDC1 will be suspended in saline with 5% human serum albumin (HSA) and placed in sterile syringes for administration to the patient. Each syringe will be labeled with a custom-designed label, identifying the patient and the vaccine.

Intranodal and intradermal vaccines will be delivered as single injections.

8.1.4 Supplier

The DC vaccine will be prepared by the cGMP facility of the University of Pittsburgh Cancer Institute, in a process analogous to the vaccine for colorectal cancer patients covered by the existing BB-IND 13,234.

8.2 Celecoxib

A sulfa non-steroidal anti-inflammatory drug (NSAID) used in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis.

8.2.1 Other names

Celebrex, Celebra, or Onsenal *(commercially available)*

8.2.2 Formulation and packaging

Celecoxib as capsules in the following dosages: 100 mg, 200 mg, and 400 mg.

8.2.3 Preparing and dispensing

Externally prepared and dispensed per treating physician prescription.Drug storage and accountability

Store at room temperature at 77 °F (25 °C) away from light and moisture. Brief storage between 59-86 °F (15-30 °C) is permitted.

8.3 Interferon-α2b

An antiviral drug approved around the world for the treatment of chronic hepatitis C, chronic hepatitis B, hairy cell leukemia, chronic myelogenous leukemia, multiple myeloma, follicular lymphoma, carcinoid tumor, and malignant melanoma.

8.3.1 Other names

Interferon-α2b, Intron® A, IFN-α2b, IFNα, IFN, NSC #377523 *(commercially available)*

8.3.2 Formulation and packaging

50 MU/mL, lyophilized powder, which must be reconstituted prior to administration.

Vial size: 10 MU/vial

Diluent: Compatible with normal saline, Ringer's injection, lactated Ringer's, and 5% sodium bicarbonate injection. IFNα should be reconstituted with 1 mL to reach a final concentration of 10:1. IV dose should be diluted in sodium chloride 0.9%/100 mL (100,000 U/mL) and given over 20 minutes. The final concentration of INTRON A should not be less than 10 million IU/100 mL

Source: Schering Plough Corp.

8.3.3 Preparing and dispensing

The lyophilized product is reconstituted as directed by the manufacturer. Investigational Drug Services will prepare and dispense.

For IV injection, it is recommended that IFNα be administered as a 100,000 U/mL solution to minimize adsorption of the drug to glass and plastic containers.

8.3.4 Concomitant medications

Interactions between IFNα and other drugs have not been fully evaluated. Caution should be exercised when administering IFNα therapy in combination with other potentially myelosuppressive agents such as zidovudine. Concomitant use of IFNα and theophylline decreases theophylline clearance, resulting in a 100% increase in serum theophylline levels. Concomitant IFNα and REBETOL (Ribavirin) use is contraindicated.

For more information on concomitant medications reference the Investigator's Brochure or package insert.

8.4 Rintatolimod (poly IC analog)

A substituted double stranded polyribonucleic acid (polyI:polyC_{12}U), rintatolimod preserves activity of polyIC with a much improved systemic toxicity profile. The product has been studied extensively for use as a vaccine adjuvant and for its direct antiviral activity, as well in several cancer studies as a monotherapy, but most extensively in chronic fatigue syndrome (CFS).

8.4.1 Other names

Poly$IC_{12}U$, Ampligen®, poly I: polyC_{12}U; Polyinosinic:polycytidylic-polyuridylic acid; polyriboinosinic/polyribocytidylic (uridylic) acid; supplied by Hemispherx Biopharma, Inc.

8.4.2 Formulation and packaging

Rintatolimod is supplied as a liquid solution in glass bottles containing 200 mg per 80 mL. Rintatolimod is a colorless solution containing 2.5 mg/mL in physiological salts (0.15 M NaCl, 0.01 M phosphate, 0.001 M Mg++). The product does not contain preservatives or antioxidants.

8.4.3 Preparing and dispensing

A vial of rintatolimod is suitable for direct IV infusion. Investigational Drug Services will prepare and dispense. Each vial should be taken from the refrigerator and allowed to equilibrate to room temperature.

8.4.4 Drug storage and accountability

Rintatolimod should be stored at 2 to 8°C, but should be infused at room temperature. Used vials

should be accounted for and destroyed according to institutional procedure.

9. CORRELATIVE STUDIES

At indicated time points (see the study calendar, Section 10) up to 60 cc of peripheral blood will be collected and cryopreserved for potential correlative studies. For additional correlative studies, we may cryopreserve the available portions of the harvested tumors, not used for the preparation of autologous vaccines. The exact scope of the correlative studies will depend on the clinical outcomes and available funding, but may include:

- Changes in circulating subsets of immune cells (flow cytometry).
- Changes in serum immune mediators, cytokines, chemokines (Luminex, ELISAs).
- Changes in gene expression of blood cells (mRNA/Taqman assays).
- Changes in the frequencies and activity of tumor-reactive cells (ELISPOTs and ELISAs)

TTP may be correlated with the changes in the blood samples from the treated subjects. For subjects who will need palliative surgery or will demonstrate clinical response and will have accessible tumor tissue, we may obtain tumor material to analyze tumor-infiltrating $CD8^+$ T cells in the resected metastatic CRC lesions and local expression of chemokines and other inflammatory mediators. This is measured as the ratio between the CD8 (or other gene) mRNA message and the expression of the housekeeping gene HPRT, in situ hybridization [ISH], and Immunohistochemistry/immunofluorescence [ICH/IF]. Examples of additional relevant parameters include total numbers of infiltrating T cells, their CD4/CD8 ratios, frequencies of FoxP3 cells, and the expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells (CXCR3, CCR5, CCR4, CCR6, and CXCR4). Using ISH, IF, and Taqman, we may also evaluate the local expression of T_{eff}-attracting chemokines: CCR5, CXCL9, and CXCL10 and CXCL11, and MDSC/T_{reg}-attracting chemokines: CCL22 and CXCL12. Similar assays may also be performed on any available pre-treatment tumors (if such material will remain after preparation of vaccines).

These potential correlative studies may allow us to determine if any blood- or tumor-associated markers are predictive of response to treatment and thus may be used as prospective inclusion criteria or assist us in prospective decisions regarding continuation of treatment in the individual patients. They may also facilitate development of additional therapies.

Any biological samples (from tissue, leukapheresis, blood draws, etc.) not needed for this research study will be de-identified and may be used for additional cancer research outside of this clinical trial.

10. STUDY CALENDAR

Baseline/screening evaluations will be conducted within 14 days and imaging will be conducted within 28 days of leukapheresis.

There is a window of ±1 week available for scheduling treatment and/or procedures at the discretion of the Investigator/Sub-investigator. This applies also if a course is missed or a subject's treatment and/or testing day(s) need to be rescheduled due to the subject's inability to comply with the study calendar (i.e., hospitalizations, business, vacation plans, travel from long distances for study treatment, in advance of the scheduled date to allow ready access to the result(s), reduce financial burden on the subject [i.e. non-UPMC insurance coverage] or reduce travel inconvenience, illness, transportation issues, holidays, family emergencies, etc.).

Parameter	Eligibility	Surgery	Screening	Week 1* (Priming)	Week 4* (Booster Cycle 1)	Week 8* (Booster Cycle 2)	Week 12* (Booster Cycle 3)	Off treatment visit[9]
Informed Consent	X							
History and Physical Exam[1]			X	X	X	X	X	
Vital Signs[2]			X	X	X	X	X	
Adverse Event/Con Meds[10]			X	X	X	X	X	X
ECOG Performance Status			X	X	X	X	X	
CEA			X					X
Urine pregnancy test[3]			X	X[3]				
CT or MR imaging (scheduled as clinically indicated)			X[8]			X		X
CBC, platelets, differential[7]			X	X	X	X	X	
Chemistries[4,7]			X	X	X	X	X	
Leukapheresis			X					
αDC1 vaccine				X	Monday	Monday	Monday	
Chemokine modulation					Tues-Fri	Tue-Fri	Tue-Fri	
Celecoxib[5]				X	M-F	M-F	M-F	
Blood (60 cc) in vitro assays[6]				X	X	X	X	
Tissue collection		X						

*. The indicated weeks represent "target weeks" In order to avoid conflict with chemotherapy, each cycle of immunotherapy can be delayed: *see the study schema*).

1. Physical examination will be performed by the investigator/sub-investigator or their designee.
2. Vital signs (temperature, blood pressure, pulse rate, and respiratory rate) will be performed before (within 30 minutes) and after (within 30 minutes) intra-nodal vaccine doses.
3. Pregnancy test is only for WOCBP who are sexually active. Test prior to cycle 1 is to be done on day 1 and must be resulted prior to vaccination.
4. Glucose, BUN, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, alkaline phosphatase, AST, ALT, total bilirubin, amylase, lipase.
5. Celecoxib will be administered orally, at a dose of 200 mg, twice daily on vaccine and CKM study treatment days
6. Peripheral blood for potential correlative studies will be collected at the following time points: once during the pre-screening, within 60 minutes before the vaccinations of the priming cycle, and booster cycles 1 and 2; and within 60 minutes after completing booster cycle 2 on Friday. Peripheral blood for potential correlative studies may additionally be drawn within 60 minutes before the vaccination of booster cycle 3 on Monday and within 60 minutes after completing booster cycles 1 and 3 on Friday.
7. Performed within 2 weeks prior to the priming dose of vaccine and 5 business days prior to cycles 1, 2 and 3 vaccines.
8. CT or MRI imaging should be performed prior to cycle 1 if the recovery period from surgery is greater than 6 weeks.
9. At the discretion of the treating physician, the tests/procedures due to be performed at the Off Study Treatment visit may be performed on the last day of the CKM treatment for scheduling purposes.
10. Performed as routine pre-operative evaluation.
11. AEs /conmeds should be collected up to 5 days after treatment, unless considered clinically significant or serious in nature in which case they will be followed until resolution or Grade 1.

11. MEASUREMENT OF EFFECT

All Patients will undergo CT or MR prior to returning for cycle 2 of the investigational treatment (approximately week 6 or 7), at the time of the off treatment visit at MD discretion, and subsequently based upon the timing the regular care for their cancer. Response or progression will primarily be evaluated by CT or MR imaging of tumor nodules using RECIST criteria v1.1, however tumor measurements are not required as patients must be completely surgically resected to be eligible.

11.1 Disease Parameters (May be used as a guideline for scan review prior to Cycle 2)

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with CT or MR imaging (CT/MR slice thickness no greater than 5 mm). . Malignant lymph nodes may be considered measureable disease if they are ≥ 15 mm in short axis as measured by CT or MR imaging with no greater than 5 mm slice thickness.

Non-measurable disease: All other lesions (longest diameter < 10 mm with CT or MR scan) are considered non-measurable disease. Bone lesions, ascites, leptomeningeal disease, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease are all non-measurable.

11.2 CT or MR-Imaging Response Criteria

CT or MR imaging will be performed with cuts of 5 mm or less in slice thickness contiguously. RECIST criteria v1.1 will primarily be used to assess tumor recurrence.

Progressive Disease (PD): The appearance or recurrence of one or more new lesions is considered progression.

11.3 Duration of Response

Time-to-Progression (TTP): Time-to-Progression is measured from the start of treatment until the criteria for progression are met

Duration of overall response: The duration of overall response is measured from the time of surgical resection until the first date that progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

12. DATA REPORTING / REGULATORY REQUIREMENTS

12.1 Data Safety Monitoring Plan

Sponsor, Investigator, Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events
- subject safety issues
- recruitment issues
- accrual
- protocol deviations
- unanticipated problems
- breaches of confidentiality

Minutes from the DSMB meetings are available to anyone unable to attend the center DSMB.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed twice a year rather than monthly by the disease center DSMB.

Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

12.2 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and Guidelines on Good Clinical Practice compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The Investigator (i.e., the study site principal investigator) and the University of Pittsburgh and University of Pittsburgh Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

12.3 Data Handling and Record-Keeping

The Investigator (i.e., the study site principal investigator) will maintain records in accordance with Good Clinical Practice.

The investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

12.4 Institutional Review Board (IRB) Approval

The investigator (i.e., the study site principal investigator) will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment, if applicable.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice.

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an sponsor's decision to modify the previously accepted clinical protocol, the sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of protocol changes requiring the submission of a Protocol Amendment include:

- Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.
- Any significant change in the design of the protocol (such as the addition or deletion of a control group).
- The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or AE; or the dropping of a test intended to monitor the safety of the investigational drug.

12.5 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on Guidelines on Good Clinical Practice; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and University of Pittsburgh Medical Center, Commonwealth of Pennsylvania, and applicable federal agencies.

12.6 Informed Consent

The investigator (i.e., the study site principal investigator) will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator, or a sub-investigator(s) designated by the sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator or sub-investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

13.1.1 Primary endpoint

- (For Phase 1) Define Recommended Phase 2 Dose (RP2D)

- To evaluate time-to-progression (TTP) in patients with peritoneal surface malignancies (PSM), including but not limited to malignant peritoneal mesothelioma and peritoneal carcinomatosis (PC) of appendiceal and colorectal primary tumors. For patients who will be completely resected, time-to-progression will be equal to recurrence.

13.1.2 Secondary endpoints

- To estimate overall survival (OS).
- Correlative studies to characterize sequential immune response changes by quantitative measures of immune cellular phenotypes and chemokine patterns in peripheral blood (see Section 9). These results will be correlated with TTP, PFS, and OS.

Note the additional discussion of the Phase 1 dose-finding studies described in Section 5.1.

13.2 Power and Sample Size for the Study of Efficacy

Based on information from our prospective database at the University of Pittsburgh over the last 10 years, we expect to treat ~ 115 patients per year (malignant peritoneal mesothelioma ~ 15 cases/year; appendiceal peritoneal carcinomatosis ~ 40 cases/year; colorectal carcinomatosis ~ 60 cases/year). We anticipate approximately 50% of treatment patients will participate in the clinical trial. A review of the literature demonstrates median time-to-progression of 20 months, 20 months and 12 months for malignant peritoneal mesothelioma, appendiceal peritoneal carcinomatosis, and colorectal carcinomatosis, respectively. We aim to demonstrate a 25% improvement in time-to-progression (malignant peritoneal mesothelioma 25 months; appendiceal peritoneal carcinomatosis 25 months; and colorectal carcinomatosis 15 months). We will need 168 patients over 3 years of accrual with an additional 1.5 years of follow-up after accrual ceases. This will provide 90% power to detect an overall improvement of 25% for an exponential test at alpha = 0.10.

13.3 Data Analysis

At the completion of follow-up in evaluable patients, we will estimate progression-free survival by the Kaplan-Meier method with Greenwood 90% confidence intervals. We will conduct a formal test of the hypothesis that progression-free survival is improved with the experimental therapy. This test will be a one sided exponential test at $\alpha = .10$ that the median progression-free survival is better than 25% compared to historical controls. If this test is significant we will conclude that the progression-free survival is improved compared to the historical control. Descriptive summaries will be prepared of all adverse events by grade, frequency and attribution. An assessment of the association between immune parameters and survival will be conducted. The panel of immune parameters will be tested for association with progression-free and overall survival with Cox proportional hazards regression.

13.4 Continuous Safety Monitoring During the Efficacy Phase

We will use continual Bayesian monitoring of treatment-related regimen limiting toxicities with a stopping rule that permits suspension of the trial by the investigator for review by the Data

Safety and Monitoring Committee (DSMC). We have selected a moderately vague prior probability of an RLT to conform to our prior beliefs that the probability of an RLT is unlikely to be greater than the 30% target of the dose finding phase and could be lower if no RLTs are found in Phase 1. Specifically we chose a beta prior distribution with parameters a = 1.93 and b = 4.74 which yields a distribution with a mode of .2, a mean of .29.

A stopping rule will be based on the updated posterior probability of an RLT. If the posterior probability exceeds .80 that 30% or more of the patients experience a treatment-related RLT, the study will be suspended pending review by the DMSC. In order to permit continuous monitoring of serious patient toxicities, the following table was prepared based upon cumulative observed AEs at any point in the trial and can be used to signal when either arm should be suspended and reviewed by the DSMC. Values are presented in the table in intervals of 5 or 10 patients

Number of RLTs needed to suspend the trial

Number of Patients	RLTs	P(π|R > .30)*	B(RLT)**.
5	4	0.927	0.031
10	5	0.831	0.15
15	7	0.857	0.131
20	9	0.879	0.113
25	10	0.812	0.189
30	12	0.841	0.159
35	14	0.865	0.135
40	15	0.812	0.193
45	17	0.839	0.164
50	19	0.862	0.141
60	22	0.842	0.162
70	25	0.826	0.18
80	28	0.811	0.195
90	32	0.854	0.15
100	35	0.842	0.163
110	38	0.832	0.174
120	41	0.822	0.184
130	44	0.813	0.194
140	47	0.805	0.202
150	51	0.842	0.163
160	54	0.834	0.171
168	56	0.812	0.194

* P(π|R > .30) = Posterior probability of a treatment-related RLT exceeds 30% given R, the number of observed RLTs and the prior distribution.

** B(RLT) is the binomial probability of observing at least the number of RLTs in column 2 if the underlying rate of RLT is 30%.

This table presents the minimum number of RLTs that dictate suspension of the trial in accordance with the stopping rule. For example, according to this table 5 RLTs among any of the

first 10 enrolled patients on the efficacy phase will trigger suspension of the trial.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.