Abbreviated Title: Adjuvant Tumor Lysate Vaccine
NIH Protocol #: 14C0053
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Title: Adjuvant Tumor Lysate Vaccine and Iscomatrix™ with or without Metronomic Oral Cyclophosphamide and Celecoxib in Patients with Malignancies Involving Lungs, Esophagus, Pleura, or Mediastinum

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Investigational Agents:

Drug Name:	H1299 Cell Lysates	Iscomatrix adjuvant	Cyclophosphamide	Celecoxib
IND Number:	15812			
Sponsor:	Center for Cancer Research			
Manufacturer:	Thoracic Epigenetics Laboratory, Thoracic Surgery Branch, NCI	CSL Limited/Behring	Generic	Generic
Supplier:	Department of Transfusion Medicine (DTM), NIH CC	CC Pharmacy	CC Pharmacy	CC Pharmacy

PRÉCIS

Background:

- During recent years, cancer-testis (CT) antigens (CTA), particularly those encoded by genes on the X chromosome (CT-X genes), have emerged as attractive targets for cancer immunotherapy. Whereas malignancies of diverse histologies express a variety of CTAs, immune responses to these proteins appear uncommon in cancer patients, possibly due to low-level, heterogeneous antigen expression, as well as immunosuppressive regulatory T cells present within tumor sites and systemic circulation of these individuals. Conceivably, vaccination of cancer patients with tumor cells expressing high levels of CTAs in combination with regimens that deplete or inhibit T regulatory cells will induce broad immunity to these antigens.
- In order to examine this issue, patients with primary lung and esophageal cancers, pleural mesotheliomas, thoracic sarcomas, thymic neoplasms and mediastinal germ cell tumors, as well as sarcomas, melanomas, germ cell tumors, or epithelial malignancies metastatic to lungs, pleura or mediastinum with no evidence of disease (NED) or minimal residual disease (MRD) following standard multidisciplinary therapy will be vaccinated with H1299 tumor cell lysates with Iscomatrix™ adjuvant. Vaccines will be administered with or without metronomic oral cyclophosphamide (50 mg PO BID x 7d q 14d), and celecoxib (400 mg PO BID). Serologic responses to a variety of recombinant CTAs as well as immunologic responses to autologous tumor or epigenetically modified autologous EBV-transformed lymphocytes will be assessed before and after receiving 6 vaccines.

Primary Objectives:

- To assess the frequency of immunologic responses to CTAs in patients with thoracic malignancies following vaccinations with H1299 cell lysate/Iscomatrix™ vaccines alone in comparison to patients with thoracic malignancies following vaccinations with H1299 cell lysate/Iscomatrix™ vaccines in combination with metronomic cyclophosphamide and celecoxib.

Eligibility:

- Patients with histologically or cytologically proven small cell or non-small cell lung cancer (SCLC; NSCLC), esophageal cancer (EsC), malignant pleural mesothelioma (MPM), thymic or mediastinal germ cell tumors, thoracic sarcomas, or melanomas, sarcomas, or epithelial malignancies metastatic to lungs, pleura or mediastinum who have no clinical evidence of active disease (NED), or minimal residual disease (MRD) not readily accessible by non-invasive biopsy or resection/radiation following standard therapy completed within the past 56 weeks.
- Patients must be 18 years or older with an ECOG performance status of 0–2.
- Patients must have adequate bone marrow, kidney, liver, lung and cardiac function.
- Patients may not be on systemic immunosuppressive medications at time vaccinations commence.

Design:

- Following recovery from surgery, chemotherapy, or chemo/XRT, patients with NED or MRD will be vaccinated via deep subcutaneous (SQ) injection with H1299 cell lysates and Iscomatrix™ adjuvant monthly until 6 vaccinations have been given.
- Vaccines will be administered with or without with metronomic oral cyclophosphamide and celecoxib.
- Systemic toxicities and immunologic response to therapy will be recorded. Pre and post vaccination serologic and cell mediated responses to a standard panel of CT antigens as well as autologous tumor cells (if available) and EBV-transformed lymphocytes will be assessed before and after vaccination.
- Numbers/percentages and function of T regulatory cells in peripheral blood will be assessed before, during, and after vaccinations.
- Patients will be followed in the clinic with routine staging scans until disease recurrence.
- The trial will randomize 28 evaluable patients per arm to either receive vaccine alone or vaccine plus chemotherapy in order to have 80% power to determine if the frequency of immune responses on the combination arm exceeds that of the vaccine alone arm, if the expected frequencies of immune responses on the two arms were 20% and 50%, using a one-sided 0.10 alpha level Fisher's exact test.
- Approximately 60 patients will be accrued to this trial.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To assess the frequency of immunologic responses to CTAs in patients with thoracic malignancies following vaccinations with H1299 cell lysate/Iscomatrix™ vaccines alone in comparison to patients with thoracic malignancies following vaccinations with H1299 cell lysate/Iscomatrix™ vaccines in combination with metronomic cyclophosphamide and celecoxib.

1.1.2 Secondary Objectives

- To examine if oral metronomic cyclophosphamide (CP) and celecoxib therapy diminishes the number and percentage of T regulatory cells and diminishes activity of these cells in patients with thoracic malignancies are at risk of recurrence.
- To examine if H1299 cell lysate/Iscomatrix™ vaccination enhances immunologic response to autologous tumor or epigenetically-modified autologous EBV-transformed lymphocytes.

1.2 BACKGROUND AND RATIONALE

Primary thoracic malignancies account for nearly 200,000 deaths annually in the United States [1]. Presently, less than 15% of patients presenting with lung and esophageal cancers or malignant pleural mesotheliomas (MPM) can be cured despite aggressive multi-modality intervention [2-4]. Median survivals for patients undergoing resection or chemo-radiation therapy of locally-advanced lung and esophageal cancers range from 18-24 months, with 5-year survival rates of 25-30% [5, 6]. Median survivals of MPM patients undergoing surgery with adjuvant chemotherapy or radiation range from 12-22 months [7]. Median survivals of patients undergoing resection of locally-advanced thymomas/thymic carcinomas range from 24-36 months [8], whereas median

survivals of patients undergoing multi-modality therapy of primary thoracic sarcomas range from 8-30 months [9, 10].

Nearly one-third of all patients dying from extra-thoracic malignancies including sarcomas and melanomas, as well as gastrointestinal, adrenocortical or genitourinary cancers develop life threatening metastases to lungs, pleura or mediastinum. Many individuals, particularly those with osteo- or soft tissue sarcomas, have pulmonary disease as the sole site of distant metastases [11, 12]. Thoracic metastasectomy appears to improve survival in properly selected patients with these malignancies (**Table 1**); in general, patient outcomes correlate with histology and stage of the primary tumors, disease free intervals, number of metastases and completeness of resection [12-19]. However, the majority of patients undergoing thoracic metastasectomy ultimately succumb to inoperable recurrent disease, due to emergence of chemo-resistant cancer stem cells [20-23].

Table 1: International Registry of Lung Metastases

Group	Resectable	Risk Factors	Median Survival (mo)
I	Yes	None: (DFI ≥36 months, single metastasis)	61
II	Yes	1: (DFI <36 months *or* multiple metastases)	34
III	Yes	2: (DFI <36 months *and* multiple metastases)	24
IV	No		14

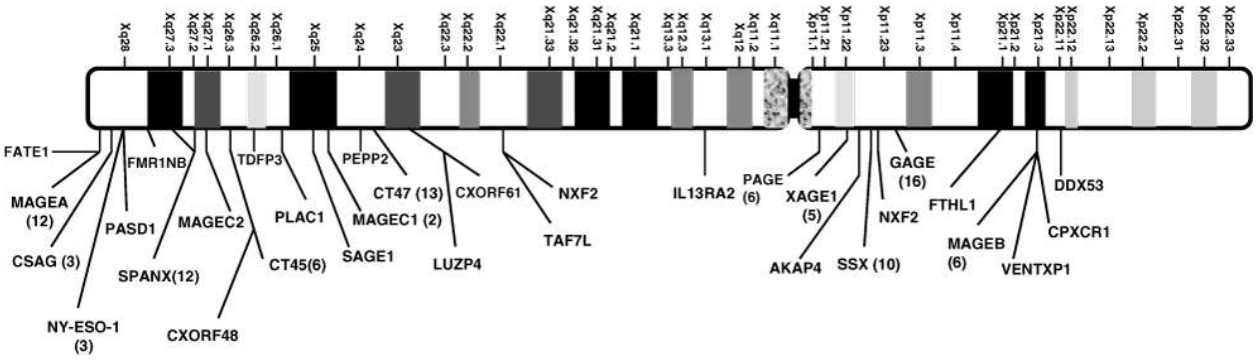
Note. Data from the International Registry of Lung Metastases.[17] Multivariate analysis showed a better prognosis for patients with germ cell tumors compared to epithelial tumors, resectable tumors, DFIs of 36 months or more, and single metastases.

Copied from ref [13].

1.2.1 Rationale for Targeting Cancer-Testis Antigens in Thoracic Malignancies

Epigenetic alterations during malignant transformation facilitate de-repression of a variety of genes, which normally exhibit stage-specific expression during germ cell development in testes or ovary [24-27]. Aberrant activation of these cancer-testis (CT) genes in somatic cells results in expression of highly restricted tumor antigens that induce serologic as well as cell-mediated immune responses in cancer patient; as such, cancer-testis antigens (CTAs) have emerged as attractive targets for cancer immunotherapy [1, 27]. To date, more than 100 CTAs have been identified, approximately half of which are encoded on the X chromosome (**Supplementary Table A**; **Figure 1**). CT-X chromosome (CT-X) genes are normally expressed in spermatogonia, and typically comprise extended families associated with inverted DNA repeats. The non-X CT genes tend to be expressed during later stages of germ cell differentiation (i.e. spermatocytes), and do not appear to be associated with extended families or inverted repetitive DNA sequences [28], [29, 30]. Relative to autosomal CT genes, CT-X genes are more frequently activated in cancer cells, and particular gene families appear to be coordinately de-repressed in a tumor-specific manner (**Supplementary Table B**).

Figure 1: Location of CT genes on X chromosome, with corresponding number of family members in parenthesis. Copied from ref [31].



Recent studies suggest that de-repression of CT-genes during malignant transformation recapitulates epigenetic reprogramming in normal pluripotent cells [27, 28, 30, 31], and that direct or functional interactions of CT antigens contribute to the malignant phenotype of cancer cells. For example, MAGE-C1 directly binds to NY-ESO-1[32]. BORIS activates NY-ESO-1 and MAGE-A1, and enhances telomerase expression in cancer cells [25, 33, 34]. Multiple MAGE family members including MAGE-A3 and MAGE-A2 complex with Kap-1, a co-repressor of p53; knock-down of MAGE-A3 or MAGE-A2 increases p53 expression and induces apoptosis in cancer cells [35, 36]. GAGE-7C confers resistance to apoptosis mediated by Fas ligand, IFN-γ, paclitaxel and radiation [37]. MAGE-C1 and MAGE-A3 enhance resistance to spontaneous or chemotherapy-induced apoptosis in myeloma cells [38]. A variety of cancer testis genes modulate the phenotype of glioblastoma stem cells [39]. Consistent with these findings, SSX enhances pluripotency and self-renewal of undifferentiated mesenchymal stem cells, as well as migration of melanoma cells [40].

In general, the frequency and magnitude of CT-X gene de-repression in human cancers coincides with advanced stage of disease. Typically, tumor samples with CT-X mRNA copy numbers >10% of testes exhibit corresponding proteins detectable by immunohistochemistry techniques. In contrast, tumors with CT-X mRNA levels < 1% of testes do not have detectable antigen expression. With the exception of synovial sarcomas or Hodgkin Lymphomas that tend to exhibit homogeneous expression of NY-ESO-1 and CT-45, respectively, the majority of human cancers exhibit extremely focal CTA expression. These observations, together with recent reports demonstrating high level CT-X gene expression in normal and malignant germ cells [28, 41, 42], as well as glioma and melanoma stem cells [43, 44], raise the possibility that CT-X antigens are preferentially expressed in pluripotent tumor cells. As such, immunotherapies targeting CT-X antigens may be novel strategies to eradicate cancer stem cells promoting systemic metastases [45-48].

1.2.2 Vaccination Strategies Targeting CT-X Antigens

Relatively limited information is available regarding the potential efficacy of vaccines targeting CT-X antigens in cancer patients. In general, immunologic responses to these vaccines are more evident in patients with no evidence of disease or minimal disease burden relative to patients with extensive, bulky disease [49]. Atanackovic et al [50] observed that a vaccine containing recombinant whole protein MAGE-A3 with a proprietary immunostimulant (AS15 or AS02B) induced $CD4^+$ and $CD8^+$ immune responses in lung cancer patients. Furthermore, a Phase II trial suggested that this vaccine might prolong survival in certain subsets of lung cancer patients undergoing definitive resection [51]. Immunologic responses in lung cancer patients coincided

with an 84 gene signature identified in tumor tissues of melanoma patients responding to MAGE-A3 vaccines [51]; the majority of genes predictive of response to vaccines were immune-related. In another trial, NY-ESO-1 DNA vaccines mediated $CD4^+$ T cell responses in 5 of 5 patients with advanced stage NSCLC, three of whom developed $CD8^+$ T cell responses to purified NY-ESO-1 peptides [52]. Two lung cancer patients with measurable disease at the start of vaccination exhibited stable disease for 16 and 23 months; two additional lung cancer patients and one esophageal cancer patient with Stage IV disease who were NED at the time of protocol entry remained free of disease.

One potential strategy to circumvent limitations of immunotherapy for cancers exhibiting low-level intra-patient as well as intra-tumoral heterogeneity of CT-X antigen expression, and the lack of recombinant polyvalent vaccines, involves immunization of patients with autologous cells genetically engineered to express high-levels of these potential targets. Fontana et al [53] observed immunologic responses to MAGE-A3 in 3 of 10 melanoma patients vaccinated with autologous PBL genetically-modified to express this CTA. In a more recent study, Russo et al [54] observed immunologic responses to MAGE-A3 in 6 of 23 melanoma patients receiving autologous lymphocytes genetically engineered to express this CTA. Of 19 patients with measurable disease, one had an objective response, and four patients exhibited durable stabilization of disease.

An alternative strategy is to vaccinate patients with allogeneic cancer cells, which exhibit high level CT-X gene expression without genetic manipulation. Recent studies conducted in the Thoracic Epigenetics Laboratory, Thoracic Surgery Branch, NCI, have identified two such lines (K562-GM and H1299), which express numerous CT-genes at levels equal to or exceeding those observed in normal testes (**Table 2**). The K562-GM line was established from HLA Class I/II deficient erythroleukemia cells, which were subsequently engineered to stably express GM-CSF [55]. K562-GM cells have been evaluated extensively in cancer patients in a wide variety of treatment regimens, with minimal vaccine-attributable toxicities [56]. The H1299 line was derived from a large cell lung carcinoma [57]; to date, this line has not been used as a cancer vaccine.

Table 2: Quantitative RT-PCR Analysis of Cancer-Testis Gene Expression in Irradiated Cryopreserved K562-GM and Non-Irradiated H1299 Cells

	BORIS	BO va 3	BO va 4	NY-ESO-1	MAGE A1	MAGE A3/6	MAGE A12	MAGE C1	GAGE	GAGE1	XAGE1	SSX1	SSX2	CT-45
K562-GM	42917	26416	3917	2	0	65531	86939	26269	6415	56	261	196	540	1672
H1299	21758	8310	4273	190021	30541	89154	196330	0	26347	33328	28864	3	0	5683
Testes	10068	9722	11021	12077	10855	9452	9726	9186	10179	8747	9309	4843	60567	8758
NHBE	0	<12	0	0	0	0	0	0	0	<1	0	0	0	0

In a recent pilot study, Smith et al [58] treated 19 patients with chronic phase CML with incomplete molecular responses to imatinib (based on RT-PCR analysis of bcr-abl) with q3week K562-GM vaccinations and concurrent imatinib. Each vaccination consisted of $1x10^8$ viable, irradiated K562-GM cells administered via intradermal injection with or without concomitant topical imiquimod cream- a toll-like receptor agonist. Overall the vaccinations were well tolerated, with Grade 1-2 injection site reactions observed in 16 patients, and transient Grade 3 reactions (pain, erythema, swelling) responding to local ice packs, seen in 3 individuals. Complete molecular responses were observed in 5 patients, and major molecular responses were seen in 5 additional individuals.

Virtually all patients exhibited polyclonal antibody responses to K562-GM lysates following vaccinations. Responses to CT-X antigens were not systematically evaluated in this trial.

In an ongoing Phase II trial in the Thoracic Surgery Branch, NCI, 19 patients received K562-GM vaccines in conjunction with metronomic oral cyclophosphamide and celecoxib as adjuvant therapy following complete resection of their malignancies. 15 patients received vaccines containing $2.5x10^7$ K562-GM cell administered monthly x 6. No vaccine-related toxicities were observed. Whereas several patients exhibited increased serologic reactivity to purified CTAs following their vaccinations, none met criteria for immunologic response. As such, 5 additional patients are currently receiving monthly vaccinations using $1x10^8$ cells per immunization. No vaccine related toxicities have been observed thus far; none of these patients are evaluable for treatment response at this time.

1.2.3 Inhibiting Treg Function to Enhance the Efficacy of Vaccines Targeting CT-X Antigens

Considerable data indicate that $CD4^+$ $CD25^+$ T cells expressing the forkhead box P3 (Fox P3) transcription factor mediate immune tolerance in cancer patients [59, 60]. Fox P3 is an intracellular protein, detection of which by flow cytometry techniques requires permeabilization of cells. As such, these regulatory T (Treg) cells are typically isolated as $CD4^+$ $CD25^+$ T cells, and exhibit functional suppressor activities in co-culture experiments. Chronic inflammation within tumors facilitates conversion of $FoxP3^+$ T_H cells to a suppressive phenotype [61, 62]. Absolute numbers and percentages of Tregs in peripheral blood of cancer patients are significantly higher compared to healthy control patients, and correlate with poor survival [63, 64].

Recent studies have shown that the capacity of cancer vaccines to induce effective antitumor immunity may be abrogated by simultaneous expansion of vaccine-induced Tregs [65, 66]. Of particular relevance regarding CT-X antigens are recent observations that regulatory T cells attenuate immune responses to NY-ESO-1 vaccines in patients with melanoma as well as prostate, lung and esophageal carcinomas [49, 52]. Furthermore, increased Treg levels are negatively associated with clinical responses in cancer patients undergoing non-myeloablative chemotherapy and adoptive immunotherapy targeting NY-ESO-1 [67].

Given the suppressive role of regulatory T cells, it is conceivable that depletion or functional inhibition of these cells may prove efficacious for cancer immunotherapy [59, 60, 68-71]. The recombinant protein containing the active domain of diphtheria toxin fused to human interleukin 2 (ONTAK) has been reported to decrease or eliminate $CD4^+$ $CD25^+$ T cells in some patients with advanced cancers [72]. The CD-25 blocking antibody daclizumab, markedly decreases Tregs and facilitates reprograming of these cells to ɤ-IFN secreting T_H cells [69, 73]. Furthermore, a variety of additional biologic or pharmacologic agents including Toll-like receptor agonists and anti-CTLA-4 antibodies, as well as cyclophosphamide (CP), fludarabine and celecoxib inhibit activation or function of regulatory T cells in-vitro and in-vivo [69, 73-77].

Several studies have suggested that the timing of Treg depletion relative to active vaccination may be critical. In a murine model, depletion of T regulatory cells appeared most effective in augmenting tumor immunity when given in conjunction with, rather than before or after vaccination [78]. In contrast, a recent clinical trial demonstrated that pre-treatment with ONTAK improved immunogenicity of vaccination with RNA transfected dendritic cells thereby improving stimulation of tumor-specific T cells in patients with renal carcinomas compared to vaccine alone

[79]. To date, the use of metronomic cyclophosphamide and celecoxib in combination with vaccines targeting CT-X antigens in humans has not been reported.

1.2.4 H1299 cells

The H1299 large cell lung cancer line was established at the NCI approximately 30 years ago. This cell line was extensively characterized, and transferred to the ATCC repository (**Appendix 2**). H1299 cells are hypertetraploid (module chromosome number = 99), and exhibit high level expression of a broad range of CT-X genes due to stable amplification of the X-chromosome. Expression levels of most common CT-X genes are higher in H1299 cells compared to K562-GM cells, and exceed levels observed in normal testes (**Table 2**). Notably, NY-ESO-1 and MAGE-A1, which are not expressed in K562-GM cells are highly expressed in H1299 cells. Because H1299 cells exhibit broader and higher levels of CT gene expression than K562-GM, H1299 cells may be more effective vaccines for inducing immunity to CTAs. However, unlike MHC-deficient K562-GM, H1299 cells exhibit class I as well as class II HLA expression (A32, B40, CW02, DRb1 01.13, DRB3*02, DQb1 05.0603); consequently, H1299 cells may induce more allo-reactivity than K562-GM cells when used as whole cell vaccines.

Recently a series of experiments were performed in the Thoracic Epigenetics Laboratory, Thoracic Surgery Branch, NCI to examine if freeze-thaw lysate techniques could be used to deplete HLA proteins while preserving expression of CT-X antigens. Briefly, H1299 cells grown in the presence or absence of the DNA demethylating agent, deoxy azacytidine (DAC), were harvested, washed extensively with HBSS, and then lysed by either traditional RIPA buffer, or freeze-thaw techniques followed by centrifugation to remove cellular debris. Protein concentrations in the lysates were quantitated by standard BSA techniques. Immunoblot techniques were then used to compare levels of several CT-X antigens as well as MHC proteins in these lysates. In general, total protein yields were lower following freeze-thaw compared to RIPA buffer lysis (**Table 3**).

Table 3:Protein Yields from Freeze-thaw vs. RIPA Lysates

	Total mg of protein/10e8 cells	Total mg of protein/10e8 cells
	Exp 1	Exp 2
H1299-FT-NM	11.6	7.8
H1299-FT-DAC	14.9	9.9
H1299-RIPA-NM	16.6	11.1
H1299-RIPA-DAC	22.9	15.3

Immunoblot analysis revealed markedly decreased levels of class I MHC proteins with equal if not higher levels of CT-X antigens in freeze-thaw relative to RIPA lysates; levels of NY-ESO-1, MAGE-A1 and MAGE-A3 were considerably higher in freeze-thaw lysates from H1299 relative to RIPA lysates from K562-GM cells (**Figure 2**). DAC treatment did not augment levels of these CT-X antigens in H1299 cells. Additional immunoblot experiments demonstrated that CT-X antigen levels in freeze-thaw lysates remained stable for at least 6 months (**Figure 3**). Collectively, these experiments demonstrate that vaccines utilizing freeze-thaw lysates from H1299 cells will have markedly higher levels of NY-ESO-1 and MAGE CT-X antigens than current K562-GM cell vaccines. Furthermore, freeze-thaw techniques deplete MHC proteins in H1299 lysates. These findings provide strong rationale for the utilization of H1299 freeze-thaw lysates as a means to immunize cancer patients against a broad range of relevant CT-X antigens.

Figure 2: Immunoblot Analysis of CT Antigens in Tumor Cell Lysates-Baseline

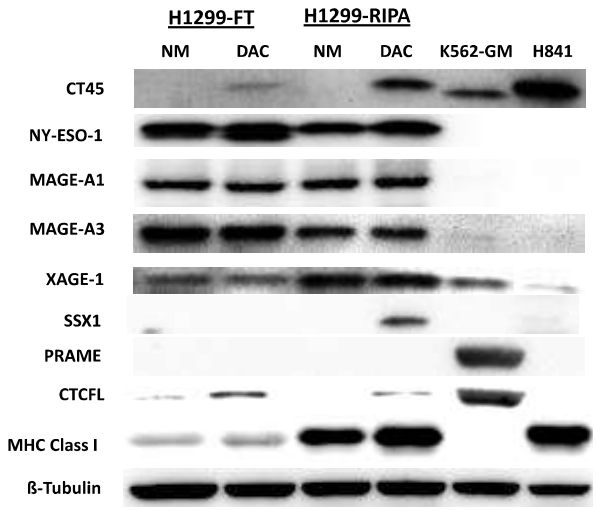
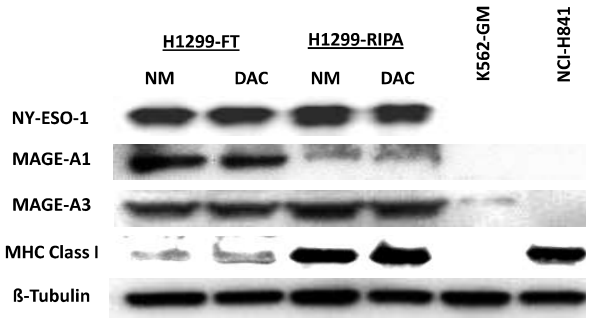


Figure 3: Immunoblot Analysis of CT Antigens in Tumor Cell Lysates-Six Months



ISCOMATRIX™ *Adjuvant*

ISCOMATRIX™ adjuvant is prepared by combining, under controlled conditions, solutions of ISCOPREP™ saponin (fractionated from an extract of the plant material of the *Quillaja saponaria* tree) with Dipalmitoyl-sn-phosphatidylcholine (DPPC) and cholesterol, in a detergent (MEGA 10), to solubilize the lipids, and buffered isotonic saline (pH 6.2). The adjuvant cage-like structures are formed during ultrafiltration to remove the detergent, and then sterilized by filtration [80, 81].

ISCOMATRIX™ adjuvant has been administered to 576 individuals in six published studies conducted by the adjuvant manufacturer involving patients with HIV or HCV, as well as healthy volunteers (**Table 4**); 286 individuals on this study did not receive any adjuvant. Demographics of volunteers in these studies are summarized in **Table 5**. Approximately 1200 additional subjects were or are currently enrolled in Phase I/II studies utilizing ISCOMATRIX™ vaccines conducted by the manufacturer's collaborators. To date, no vaccine-related deaths have been reported in any of these trials. One possibly related SAE of cardiac arrest occurred in a patient with underlying cardiac disease. The most common injection site reactions reported in the manufacturer's studies (N=576) were pain (70.8%), swelling (33.7%) and redness (33.9%). Systemic reactions included myalgia (45.1%), fatigue (32.3%) and headache (31.0%) comprising a transient flu-like syndrome. No significant changes in systemic markers of auto-immunity (anti-cardiolipin antibodies, anti-β2-glycoprotein-1 antibodies, or anti-nuclear antibodies), allergy (IgE levels), or inflammation (C Reactive Protein) have been observed (**Table 6**) [80].

Table 4: Clinical trials with optimized ISCOMATRIX™ vaccines

Study	Study Population	Number[a]	Number of Vaccinations	Vaccination Schedule	Dose		Control
					IMX (ISCO™ Units)	Antigen (µg)	
HCV Study 1	Healthy Volunteers 18-45 years (n = 30)	24	3	Days 0, 28, 56	90	5, 20, 50	PBS
HCV Study 2	HCV positive 18-60 years (n = 33)	23	3	Days 0, 28, 56	90	5, 50	PBS
HPV Study 1	Healthy Volunteers 18-45 (n = 42)	36	3	Days 0, 28, 84	45, 90	5, 25, 70, 240	PBS
HPV Study 2	HIV positive 18-60 years (n = 35)	28	3	Days 0, 28, 84 or Days 0, 14, 70	90	25, 70, 240	PBS
Influenza Study 1	Healthy volunteers 60-64 years (n = 110)	55	1	Day 0	45	15, 45	Commercial influenza vaccine or influenza antigen control
Influenza Study 2	Healthy volunteers 18-45 years and ≥60 years (n = 612)	410	1	Day 0	22.5, 45, 90	45	Commericial influenza vaccine
Total	862	576					

a. Number of subjects receiving ISCOMATRIX™ vaccines and does not include those who received placebo or antigen control vaccine

HPV: Human Papillomavirus; HCV: Hepatitis C Virus; IMX: ISCOMATRIX™ adjuvant; PBS: Phosphate-buffered saline

Table 5: Demographic characteristics of subjects exposed to ISCOMATRIX™ adjuvant

	Adjuvant Dose (ISCO™ Units)				
	0[a]	**22.5**	**45**	**90**	**Total**
Number of Subjects	286	106	173	297	862
Age (years)					
Mean	61.7	52.7	54.6	55.9	57
Median	64	62	62	61	63
Range	18-97	19-74	19-74	18-95	18-97
Gender					
Female	149 (52.1%)	67 (63.2%)	88 (50.9%)	124 (41.8%)	428 (49.7%)
Male	137 (47.9%)	39 (36.8%)	85 (49.1%)	173 (58.2%)	434 (50.3%)
Population					
Healthy adults (< 60 years)	78 (27.3%)	46 (43.4%)	58 (33.5%)	146 (49.2%)	328 (38.1%)
Elderly (≥ 60 years)	208 (72.7%)	60 (56.6%)	115 (66.5%)	151 (50.8%)	534 (61.9%)

a. Subjects in this group receive either placebo or active comparator

Table 6: Number (%) of participants with elevated markers of autoimmunity, allergy and inflammation

	Comparator N = 102			**90 ISCO™ Units IMX N = 106**		
Marker	**PRE[a]**	**POST[b]**	**NEW POST[c]**	**PRE**	**POST**	**NEW POST**
CLA	4 (3.9%)	3 (2.9%)	0 (0.0%)	10 (9.4%)	9 (8.5%)	0 (0.0%)
B2G	1 (1.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
ANA	20 (19.6%)	19 (18.6%)	0 (0.0%)	11 (10.4%)	12 (11.3%)	1 (1.0%)
IgE	18 (17.6%)	16 (15.7%)	1 (1.0%)	23 (21.7%)	21 (19.8%)	0 (0.0%)
CRP	7 (6.9%)	5 (4.9%)	1 (1.0%)	4 (3.8%)	4 (3.8%)	1 (1.0%)

a. PRE = participant with out of reference range value pre-vaccination

b. POST = participant with out of reference range value post-vaccination

c. NEW POST = participant with normal value pre-vaccination and an out of reference range value post-baseline

IMX: ISCOMATRIX™ Adjuvant, CLA: anti-cardiolipin antibodies; B2G: anti-β_2-glycoprotein-1 antibodies; ANA: anti-nuclear antibodies, IgE Total IgE, CRP: C Reactive Protein

Recent studies indicate that ISCOMATRIX™ adjuvant accelerates and potentiates immune responses to a variety of vaccines containing viral antigens derived from HCV, HPV, and HIV [80, 81]. Compared to traditional adjuvants such as aluminum hydroxide, ISCOMATRIX™ induces markedly higher vaccine-mediated antibody responses, thereby enabling use of lower doses of antigen per vaccine [82]. Furthermore, ISCOMATRIX™ vaccines have been shown to induce CD4(+) and CD8(+) T cell responses to a variety of antigens in human studies [81]. ISCOMATRIX™ induces rapid innate immune responses at injection sites and enhances cross-priming of $CD8^+$ T cells [83, 84]. Of particular relevance regarding this protocol are observations that ISCOMATRIX™ adjuvant enhances cross-presentation of tumor antigens such as NY-ESO-1 to induce significant, long-lasting $CD4^+$ and $CD8^+$ T cell responses in cancer patients [80, 85]. Persistence of cell mediated immunity to NY-ESO-1 has not been observed in cancer patients receiving vaccinations with NY-ESO-1 protein alone [86]. Furthermore, ISCOMATRIX™ adjuvant enhances humoral immune responses to NY-ESO-1 [87]. Recent reports that regulatory T cells attenuate cell-mediated immune responses to NY-ESO-1/ISCOMATRIX™ vaccines [49], together with observations that these vaccines induce expansion of NY-ESO-1-specific Tregs provide strong rationale for the use of ISCOMATRIX™ in conjunction with strategies to deplete/inhibit Tregs as a means to enhance immunologic responses to vaccines targeting CTAs.

1.2.5 Cyclophosphamide (CP)

CP has been extensively evaluated in cancer patients. This alkylating agent mediates immunomodulatory and antiangiogenic effects at low doses; at higher doses, CP induces significant cytotoxicity in cancer cells of various histologies. Several recent studies have examined the toxicities and potential clinical efficacy of low dose metronomic cyclophosphamide [88]. Nelius et al [89] observed no Grade 3/4 toxicities, in 17 patients with docetaxel-resistant hormone-refractory prostate cancer receiving metronomic CP (50mg PO qd) with dexamethasone. Nine patients exhibited PSA responses, and five patients noted decreased bone pain while receiving this treatment regimen. No patient exhibited clinically significant hematologic toxicities. Additional studies have demonstrated clinical activity with minimal CP-attributable toxicities in patients with advanced ovarian or breast cancers receiving oral metronomic CP (50 mg/d) in combination with bevacizumab or capecitabine/bevacizumab, respectively [90, 91]. Moderate lymphopenia was observed in several patients.

Ghiringhelli et al [76] examined the immunomodulatory effects of metronomic oral CP (50 mg bid x 7d q 14d) in 9 patients with advanced malignancies. $CD4^+$ $CD25^+$ T regulatory cells in peripheral blood of these cancer patients were significantly higher than healthy volunteers. Metronomic CP therapy dramatically decreased total numbers, and percentages of Tregs in these patients (28.7 +/- 9.4 vs 6.4 +/- 5.4 cells/mm^3, $p<0.0001$; 7.9 +/- 1.5% vs 3.1 +/-1.8%, $p<0.0001$, respectively). Selective depletion of Tregs was dose-dependent, as higher doses of CP (200 mg/d) depleted all lymphocyte populations in an additional six patients.

Laheru et al [92] vaccinated 50 pancreatic cancer patients with allogeneic pancreatic cancer cells expressing GM-CSF, twenty of whom received CP (250 mg IV) one day prior to their vaccinations. $CD8^+$ T-cell responses to mesothelin were observed predominantly in patients receiving vaccine plus CP. In a more recent Phase II trial, Camisaschi et al [93] randomized patients undergoing resection of locally advanced melanomas to receive intermittent low dose IV CP, sub Q IL-2, and four tumor derived peptides or observation. Several weeks following treatment, regional lymph nodes were excised; Tregs and inhibitory (TGF-β and IL-10) cytokine levels were significantly lower in lymph nodes from patients receiving CP compared to control patients. Of particular note,

no autoimmune toxicities were observed in patients receiving metronomic CP therapy with or without cell vaccines.

In a large Phase III trial, melanoma patients were randomized to receive CP (200 mg/m^2 IV) or no pretreatment prior to administration of 12 class I MHC-restricted melanoma peptides. CP did not enhance CD4 or CD8 T cell responses to the vaccines or improve outcome [94]. Collectively, these studies suggest that the effects of CP on Treg levels and immunologic responses to cancer vaccines may be contingent on dose and timing of CP administration, and type of vaccines.

1.2.6 Celecoxib

Cyclo-oxygenase is the rate-limiting enzyme for the conversion of arachidonic acid to prostaglandin and thromboxane. Two isoforms of COX enzymes have been described: COX-1, which is constitutively expressed in most normal tissues, and COX-2, which is induced in normal cells by a variety of inflammatory mediators and growth factors, and is overexpressed in many human cancers [95-97].

COX-2 expression enhances resistance to apoptosis, promotes angiogenesis via up-regulation of VEGF, FGF, TGF-alpha and beta, as well as IL-8 in tumor tissues, and facilitates invasion/metastasis of cancer cells [95, 97-99]. Tumor cells secrete IL-1 which induces COX-2 expression in mesenchymal stem cells within the tumor microenvironment to activate β-catenin signaling and promote a mesenchymal-stem-like phenotype in adjacent cancer cells [100]. In addition, COX-2 expressed by cancer cells or adjacent stromal tissues modulates IL-10/IL-12 ratios resulting in increased IL-10 levels, which potentiate the suppressive function Tregs present in tumor tissues as well as systemic circulation of cancer patients [99, 101-105]. Furthermore, COX-2 expression in the tumor microenvironment markedly inhibits dendritic cell function [99]. Recent studies indicate that PGE-2 mediated expression of indoleamine 2, 3,-dioxygenase (IDO) in dendritic cells correlates with activation of Tregs and a tolerogenic phenotype [61, 106, 107]. The selective COX-2 inhibitor, celecoxib decreases IDO expression in dendritic cells, and diminishes Treg activation in murine lung cancer models [77]. Furthermore, celecoxib in combination with MUC-1 vaccines inhibits progression to cancer in a transgenic murine pancreatic cancer model, and markedly enhances the efficacy of dendritic cell vaccines in transgenic mice developing spontaneous breast cancers via inhibition of IDO expression and diminution of Treg function [108, 109].

Celecoxib has been used extensively in patients with arthritis and trauma, and until recently, was under evaluation as a cancer chemoprevention agent. Long-term use of celecoxib as a chemoprevention agent may be limited due to concerns that this drug may increase the risk of cardiovascular events in patients with pre-existing atherosclerotic heart disease [110]; however, a recent meta-analysis revealed no evidence that celecoxib increases the relative risk of cardiovascular disease [111]; furthermore, there are no data indicating that relatively short-term use of celecoxib is deleterious in cancer patients. Plasma concentrations achievable in humans following 400 mg PO BID dosing of celecoxib approximate 5uM; this dose of celecoxib is sufficient to significantly reduce COX-2 activity and diminish PGE-2 levels in lung cancer patients [112, 113].

1.2.7 Metronomic Cyclophosphamide (CP) and Celecoxib

Growth of tumor metastases are dependent on neoangiogenesis, which is mediated in part by mobilization of bone marrow-derived endothelial progenitor cells (EPC). Preclinical studies have

demonstrated that low dose metronomic CP in combination with agents targeting VEGF signaling induce potent antitumor activity in breast and melanoma xenografts [114]. Particularly relevant to this protocol are observations that metronomic CP in combination with Piroxicam (a non-selective COX inhibitor) significantly delays tumor recurrence in dogs with incompletely resected sarcomas [115]. Clinical trials have also suggested that metronomic CP and celecoxib may be beneficial in cancer patients. Stoelting et al [116] observed that metronomic trofosfamide with or without celecoxib statistically decreased circulating EPC in 18 adolescents with refractory solid tumors. Andre et al [117] observed disease stabilization lasting >20 months in 7 of 17 pediatric patients with refractory advanced malignancies receiving metronomic CP and celecoxib. Stempak et al [118] observed durable disease stabilization in 4 of 33 patients with advanced cancers following treatment with either metronomic vinblastine or CP with celecoxib. In all of these trials metronomic therapy was well tolerated.

Buckstein et al [119] examined response rates and toxicities in 35 patients with relapsed, refractory lymphoma receiving metronomic CP (50 mg PO qd) with celecoxib (400 mg PO BID). The overall response rate was 37%. The most common complication was skin rash (40%) attributable to celecoxib; myelosuppression and GI toxicities were infrequent. Three patients developed deep vein thromboses, twelve patients underwent transient (1 – 2 month) dose reductions of celecoxib, CP or both due to Grades 2 – 3 rash (n=4), Grades 2 – 3 LFTs (n=2), Grades 3 – 4 neutropenia (n=2), or Grade 3 thrombocytopenia (n=2). Collectively, these data indicate that metronomic oral CP/celecoxib was active, and well tolerated in these heavily pretreated lymphoma patients.

In an ongoing Phase II trial in the Thoracic Surgery Branch, NCI, 20 patients with either completely resected primary thoracic malignancies or extra thoracic neoplasms metastatic to the chest have received irradiated, viable K562-GM vaccines ($2x10^7$ or $1x10^8$ cells per vaccination) administered in conjunction with oral metronomic cyclophosphamide (50 mg PO BID x7d q14d) and celecoxib (400 mg PO BID), which commenced one week prior to the first vaccination. Metronomic chemotherapy has been well tolerated, with dose reductions required only in one patient with gastric bypass (nausea), and one elderly woman with small stature who developed asymptomatic hyperkalemia. Whereas local injection site reactions have been noted in most of the patients, ELISAs have demonstrated minimal serologic reactivity to a panel of CT-X antigens in 9 evaluable patients following the six months immunization period. Collectively, these preliminary findings demonstrate that allogeneic tumor cell vaccines are well tolerated in the context of metronomic cyclophosphamide and celecoxib, and suggest that CTA levels within the K562-GM vaccines administered to the first 12 (three of whom were not evaluable for response) patients were insufficient to induce immunity to CT-X antigens.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients with histologically or cytologically proven lung or esophageal cancers, thymic or mediastinal germ cell tumors, malignant pleural mesotheliomas, or primary thoracic sarcomas, as well as patients with sarcomas, melanomas, germ cell tumors, or epithelial malignancies metastatic to the lungs, mediastinum, or pleura that have no clinical

evidence of active disease (NED) or minimal residual disease (MRD) not readily accessible by non-invasive biopsy or resection/radiation following standard therapy.

2.1.1.2 Diagnosis must be confirmed by the NCI Laboratory of Pathology.

2.1.1.3 Patients must be enrolled within 56 weeks following completion of therapy.

2.1.1.4 Patients must have completed standard therapy for their malignancy and recovered from all toxicities to less than or equal to Grade 2 within 3 weeks prior to enrollment.

2.1.1.5 Patients with intracranial metastases, which have been treated by surgery or radiation therapy, may be eligible for study provided there is no evidence of active disease and no requirement for anticonvulsant therapy or steroids following treatment.

2.1.1.6 Patients must have an ECOG performance status of 0 – 2 (See **Appendix 3**).

2.1.1.7 Patients must be 18 years of age or older due to the unknown effects of immunologic responses to this vaccine during childhood and adolescent development.

2.1.1.8 Patients must have evidence of adequate bone marrow reserve, hepatic and renal function as evidenced by the following laboratory parameters:

- Absolute neutrophil count greater than 1500/mm^3
- Platelet count greater than 100,000/mm^3
- Hemoglobin greater than 8 g/dl (patients may receive transfusions to meet this parameter)
- PT within 2 seconds of the ULN
- Total bilirubin <1.5 x upper limits of normal
- Serum creatinine less than or equal to 1.6 mg/mL or the creatinine clearance must be greater than 70 mL/min/1.73$m^{2.}$

2.1.1.9 Seronegative for HIV antibody. Note: The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive may have decreased immune competence and thus may be less responsive to the experimental treatment.

2.1.1.10 Seronegative for active hepatitis B, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.11 The effects of the study treatment on the developing human fetus are unknown; thus, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) within 28 days prior to study entry, for the duration of study participation and up to 120 days after the last dose of the drug. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.12 Patients must be able to understand and willing to sign an informed consent.

2.1.1.13 Ability and willingness to co-enroll on the screening and tissue collection protocol 06C0014, "Prospective Evaluation of Genetic and Epigenetic Alterations in Patients with Thoracic Malignancies".

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are initially rendered NED or have MRD following standard therapy but exhibit disease progression prior to initiation of vaccination.

2.1.2.2 Patients requiring chronic systemic treatment with steroids.

2.1.2.3 Patients receiving warfarin anticoagulation, who cannot be transitioned to other agents such as enoxaparin or dabigatran, and for whom anticoagulants cannot be held for up to 24 hours.

2.1.2.4 Patients with uncontrolled hypertension (>160/95), unstable coronary disease evidenced by uncontrolled arrhythmias, unstable angina, decompensated CHF (>NYHA Class II), or myocardial infarction within 6 months of enrollment.

2.1.2.5 Patients with other cardiac diseases may be excluded at the discretion of the PI following consultation with Cardiology consultants.

2.1.2.6 Patients with any of the following pulmonary function abnormalities: FEV, < 30% predicted; DLCO < 30% predicted (post-bronchodilator); oxygen saturation less than 92% on room air.

2.1.2.7 Female patients who are pregnant or breastfeeding. Because there is unknown, potentially harmful effects of immune response to CT-X antigens and stem cell proteins that may be expressed in placenta, fetus, and neonates.

2.1.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations 3 months prior to enrollment that would limit compliance with study requirements.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Participants may also be recruited through self-referrals, physician referrals, and referrals from the NIH Clinical Center (CC) Office of Patient Recruitment.

2.1.4 Screening Evaluation

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol 06C0014 (Prospective Evaluation of Genetic and Epigenetic Alterations in Patients with Thoracic Malignancies). The following screening activities will be performed after the consent for screening has been signed:

Within 4 Weeks (+5 Days) Prior to Initiation of Treatment

a. Confirmation of histology by the Laboratory of Pathology (may be more than 4 weeks prior to vaccination). This confirmation may be done under protocol 06C0014 (patients may be co-enrolled).

b. HLA class I and class II analysis of PBMC (may be more than 4 weeks prior to initiation of treatment).

c. Screening 12-lead EKG. Patients with EKG evidence of cardiac ischemia (ST depression/elevation greater than or equal to 2mm), or arrhythmia will undergo cardiology evaluation to determine eligibility.

d. Contrast enhanced CT scans of chest, abdomen, pelvis, as well as brain MR and nuclear medicine scans (if indicated by clinical parameters) to evaluate the status of disease (all imaging studies must be obtained 4 weeks prior to vaccination).

e. Pulmonary function tests

f. Fecal occult blood test

g. Viral markers protocol screen (HBsAg, anti HCV, anti-HIV) (may be more than 4 weeks prior to initiation of treatment)

Within 2 Weeks (+5 Days) Prior to Initiation of Treatment

a. Complete history and physical examination including assessment of vital signs and ECOG status

b. Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid

c. CBC, differential, platelet count, PT, PTT

d. Urinalysis and culture if indicated.

Within 2 Calendar Days Prior to the Start of Treatment

a. Women of child-bearing potential will have a urine or serum βhCG pregnancy test

2.2 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2: CCR Participant Registration & Status Updates found here.

2.2.1 Treatment Assignment and Randomization/Stratification Procedures (For Registration Purposes Only):

2.2.1.1 Cohorts

Number	Name	Description
1	Cohort 1	Subjects with thoracic cancers, sarcomas, melanomas, germ cell tumors, or epithelial malignancies metastatic to the lungs, mediastinum, or pleura.

2.2.1.2 Arms

Number	Name	Description
1	Vaccine plus chemotherapy	H1299 cell lysate vaccine with metronomic chemotherapy
2	Vaccine alone	H1299 cell lysate vaccine

2.2.1.3 Stratifications, Randomization and Arm Assignment

There is one stratification factor in the randomization: thoracic vs. non-thoracic primary sites of disease.

Name	Distinct Options
Thoracic primary site of disease	• yes • no

Within each of these two stratification groups patients will be randomized at study entry to either Arm 1 or Arm 2. Arm 1 will receive treatment with H1299 cell lysate vaccine with metronomic chemotherapy and Arm 2 will receive treatment with H1299 cell lysate vaccine alone. The Central Registration Office will be contacted via encrypted email at: ncicentralregistration-l@mail.nih.gov to determine the appropriate assignment for each patient. Once the NCI Central Registration Office receives subject stratification group assignment, randomization to treatment arms will be performed by the CRO.

3 STUDY DESIGN

This is a Phase 1/2 first in human study to assess the immunologic efficacy of an H1299 cell lysate vaccine. Patients potentially eligible for trial will be initially evaluated, and undergo subsequent eligibility assessment in the Thoracic Surgery Clinic, NCI. Up to 60 patients deemed eligible for vaccination will be randomized on a 1:1 basis to receive either H1299 lysate/ISCOMATRIX™ vaccine with metronomic chemotherapy, or H1299 lysate/ISCOMATRIX™ vaccine alone. Due to the potential heterogeneity of patients eligible for study, randomization will simply stratify patients based on thoracic vs. non-thoracic primary sites of disease, rather than specific histologies.

3.1 DRUG ADMINISTRATION

3.1.1 H1299 Lysate Vaccine

- Vaccines will be prepared in the Thoracic Epigenetics Laboratory, Thoracic Surgery Branch, NCI, according to standard operating procedures (SOP). Briefly, freeze thaw-lysates from $1x10^8$ H1299 cells (10 mg total protein) in 0.5-1 mL PBS will be mixed with 1 mL ISCOMATRIX™ in a 2 mL syringe and placed on ice. Please see Section **14.1** for additional preparation instructions.
- Vaccines will be delivered to the outpatient clinic by a member of the Thoracic Epigenetics Laboratory. Prior to administration, the vaccine identity label will be double-checked by two authorized staff (MD or RN), and identification of the product and documentation of administration will be entered in the patient's chart, as is done for the blood banking protocols. Vaccinations will be administered by licensed medical personnel in the Thoracic Surgery Clinic.
- Patients will receive vaccinations on Day 1 (± 5 days) of each vaccine cycle (one cycle = 4 weeks). Vaccines may be delayed up to 2 weeks with the exception of those subjects whose treatment was interrupted due to product manufacturing issues; these subjects may have treatment delayed for up to 1 year. The vaccine(s) will be administered via two, 1 mL deep subcutaneous injections into the proximal extremities (preferably in the anterior thighs). The same extremity will be used for each cycle of vaccine

administration. If a patient experiences a dose-limiting toxicity (DLT) as described in Section **3.3** below, then no other vaccinations will be given to that patient.

- The first 3 patients accrued to this study will be enrolled sequentially, with a 14-day delay between initiation of treatment for each patient to monitor for acute and delayed toxicities.
- Patients will be monitored in the clinic area for 4 hours after the first vaccination to rule out acute toxicities (i.e., anaphylaxis, respiratory compromise). Vital signs will be recorded prior to injection and at 30 minutes following injection. Patients will be observed for 60 minutes following the second and third vaccinations, unless clinically indicated otherwise.
- Patients who exhibit an immunologic response to vaccination at the evaluation ~1 month after receiving 6 vaccines and who remain NED at that time, will be offered additional vaccinations as described in Section **3.6**.

3.1.2 Cyclophosphamide and Celecoxib

- Cyclophosphamide and Celecoxib will be supplied via the NIH Clinical Center Pharmacy. However, in the rare event when a patient loses his/her prescription and needs immediate resupply, a finite number of pills can be prescribed by the patient's private oncologist to allow continuation of treatment while a new supply is being sent from the NIH Clinical Center Pharmacy.
    - Cyclophosphamide will be administered at a dose of 50 mg PO BID for 7 days prior to the first dose of vaccine and then on Days 8 through 14, and 22 through 28 of each treatment cycle.
    - Celecoxib will be administered at a dose of 400 mg PO BID for 7 days prior to the first dose of vaccine and then on Days 1 through 28 of each vaccine cycle.
    - In the event that vaccines are delayed, patients will continue to take celecoxib daily and cyclophosphamide every other week.
- Patients will be dispensed a 28 day supply of medications; 28 days equals one cycle, 3 cycles equals one course. Patients will be instructed to take the medications at the **same time each day (**± 6 hours**),** with food. If a dose cannot be taken within this time frame, patients will be instructed not to make up the dose.
- Prior to discharge from the clinic, the research nurse will review the following information with the patient:
    - Expected side effects from the 3 agents
    - The signs and symptoms to report to the study team
    - How to complete the medication self-administration record and patient diary (For a sample of these documents, see **Appendix 4**)

3.2 Treatment Delays and Dose Modifications

- This study is intended to evaluate potential efficacy of H1299 lysate/ISCOMATRIX™ vaccines alone or in combination with metronomic cyclophosphamide and celecoxib.

It is anticipated that the lysate vaccines will be well tolerated based on previous trials using a similar number of whole K562-GM cells in ongoing vaccine protocols, and the safety of ISCOMATRIX™. There will be no vaccine dose modifications; patients who experience a DLT (as described in Section **3.3** below) will not receive any further treatment.

- Cyclophosphamide may be reduced to 50 mg PO daily (dose level -1) or, if necessary, 25 mg PO daily (dose level -2) for Grade 3 or greater neutropenia, lymphocyte count decreased or thrombocytopenia, or as clinically indicated, at the discretion of the Principal Investigator. No further cyclophosphamide dose reductions will be permitted, cyclophosphamide will be discontinued, and patient will continue treatment with vaccine and celecoxib alone (please see **Appendix 5** for dose modification table).
- Celecoxib may be reduced on two occasions to 200 mg PO BID (dose level -1) or, if necessary, 100 mg PO BID (dose level -2) for Grade 3 or greater nausea and vomiting, abdominal pain, or electrolyte imbalances; Grade 2 or greater dyspepsia, increase in liver enzymes or serum creatinine attributable to celecoxib, or as clinically indicated, at the discretion of the Principal Investigator. Celecoxib will be discontinued for any gastrointestinal bleed greater or equal to Grade 2 or any need for further dose reductions. In the event of celecoxib discontinuation, the patient will continue treatment with vaccine and cyclophosphamide (please see **Appendix 5** for dose modification table).
- Vaccines may be delayed up to two weeks if unrelated to vaccine-induced toxicities with the exception of those subjects whose treatment was interrupted due to product manufacturing issues; these subjects may have treatment delayed for up to 1 year. This may be necessary for out of town patients (particularly those taking cyclophosphamide and celecoxib) who for unexpected reasons cannot get to the clinic on the scheduled day (i.e. inclement weather, sickness, etc.). These patients may need to wait another 2 weeks for vaccine administration so as not to disrupt the cycling of metronomic chemotherapy.

3.3 DEFINITION OF DOSE LIMITING TOXICITY (DLT)

Patients who develop a DLT will not receive any further study treatment.

DLT is defined as follows:

- Grade 2 or greater autoimmune or hypersensitivity reaction attributable to the vaccine, or
- Grade 3 or greater toxicity, at least possibly related to the vaccine, except for Grade 3 toxicities such as local injection site reactions, fatigue, fever or local adenopathy that resolve to Grade 2 or less within 2 weeks.

3.4 PROTOCOL STOPPING RULES

The study will be halted pending discussions with the FDA and NIH Intramural IRB if the following conditions are met:

- Any autoimmune DLT at least possibly related to the vaccine that occurs in 3 of up to the first 10 patients who received study treatment, or

- Any Grade 3 or greater toxicity, at least possibly related to the vaccine, except for known toxicities such as local injection site reactions, fatigue, fever or local adenopathy.

3.5 ON STUDY EVALUATION

(See Sections **16.6.1** and **16.6.2** in **Appendix 6** for a tabular summary of trial assessments and interventions in the chemotherapy and no chemotherapy cohorts respectively.)

Within 4 Weeks Prior to the First Vaccination

- Lipid panel (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol-calculated); thyroid panel (TSH, free T4)

Within 2 Calendar Days Prior to Each Vaccination

- CBC with differential
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Serum or urine beta hCG for women of child bearing potential
- Physical examination including vital signs, pulse oximetry and ECOG status
- Review of patient diary and toxicity assessment
- Urinalysis

Within 1 Week Prior to Second Vaccination

- Fecal occult blood test (only in patients randomized to the vaccine + chemotherapy arm)

Every Week (±5 days) for the First 4 Weeks – Following the First Vaccination Only (May be Done by Home Oncologist with Results Faxed to Thoracic Surgery Office)

- CBC with differential
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK (optional), Uric Acid

Every 2 Weeks (±5 days) Between Vaccinations 2 and 3, and Vaccinations 3 and 4 (May be Done by Home Oncologist with Results Faxed to Thoracic Surgery Office)

- CBC with differential
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK (optional), Uric Acid

Every 12 Weeks (+/- 4 Weeks) During Treatment

- Research bloods as described in Section **5.1.1**

Every 24 Weeks (+/- 2 Weeks) During Treatment

- Fecal occult blood test (only in patients randomized to the vaccine + chemotherapy arm)

One Month Following the Sixth Vaccination

- Analysis of CT gene expression profile in tumor specimen (if available) by quantitative RT-PCR analysis using normal testes as control.

  Note: Additional tests may be requested at the discretion of the treating physician as clinically indicated.

Approximately 1 Month Following the First Six Vaccinations, **and** Every 6 Months During Retreatment

- Response evaluation as described in Section **6.3**.

3.6 CONTINUED TREATMENT

- One month following the sixth vaccination, patients will return to the Thoracic Surgery Clinic to undergo treatment evaluation. During this visit, the patient will have blood drawn for immunologic evaluations as per Section **6.3**. We expect immunologic evaluation results to be completed in eight to twelve weeks time. While the patient is awaiting the results of the immunologic evaluation, celecoxib and cyclophosphamide will be stopped, to avoid unnecessary exposure to these drugs in the event the patient is a non-responder. Patients responding to therapy as outlined in Section **6.3** will be immediately restarted on metronomic CP and celecoxib and will receive two additional vaccinations with H1299 lysate/ ISCOMATRIX™ at 3 months after receiving the sixth vaccine (+/- 4 weeks) and 6 months after receiving the 6th vaccine (+/- 4 weeks). Those individuals not responding to treatment as outlined in Section **6.3** will be removed from the study (refer to Section **3.8**). Patients receiving additional vaccinations will be evaluated for response at 7 months after receiving the 6th vaccine.
- Patients who do not exhibit response at the assessment 1 month after receiving six vaccines will be removed from study, and continue periodic surveillance in the clinic as defined in Section **3.8.2**.
- Prior to retreatment, patients will be evaluated as described in Section **3.5**.
- Vaccines will be administered as described in Section **3.1.1**.
- All metronomic cyclophosphamide, celecoxib and vaccines will be discontinued at 7 months after receiving 6 vaccines in responders who receive additional treatment following the first 6 vaccines.

3.7 COST AND COMPENSATION

3.7.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY, POST-TREATMENT FOLLOW-UP AND OFF STUDY CRITERIA

3.8.1 Criteria for Removal from Protocol Therapy

- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Disease progression
- Patient develops symptoms that require treatment with systemic steroids
- DLT due to vaccine (Section **3.3**)
- No evidence of immunologic response to therapy
- Investigator discretion
- Positive pregnancy test

3.8.2 Post-Treatment Follow Up

- Patients exhibiting an immunologic response may be followed for up to 5 years following the initial vaccine administration (or until either loss of immunologic response or disease progression is documented). Patients who do not exhibit an immunologic response after the initial 6 vaccines or who exhibit either loss of immunological response after initial response or disease progression will be taken off-study. These patients may either be followed under the Thoracic Epigenetics Protocol 06C0014 or be referred for additional therapy if indicated based on conventional imaging studies.
- Post treatment evaluations will be performed on all responding patients 3 months following the last injection, and then every 3 months for 1 year then every 6 months

for up to 4 years or until disease progression/loss of immunological response is documented. (All visits +/- 2 weeks). At each visit, the following will be performed:

- Physical examination including vital signs, pulse oximetry and ECOG status
- Serum ELISAs for response to CT-X and related antigens, and if possible in-vitro assessment of response to autologous tumor cells and/or DAC/DP treated EBV-transformed B lymphocytes (Note: these studies are only performed every 6 months in patients exhibiting an immunological response to the initial six vaccinations).
- Patients who are unwilling or unable to travel to the NIH Clinical Center will be contacted by phone, secure videocall or other NIH approved remote platforms (used in compliance with local policies, including Policy M20-1).

3.8.3 Off Study Criteria

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

- Participant requests to be withdrawn from study
- Completion of the treatment period (for patients not responding to treatment) or the follow up period (for patients responding to treatment)
- Death
- Lost to follow up
- PI decision to close the study
- Investigator discretion

3.8.4 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 3 business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 SUPPORTIVE CARE

Concomitant medications to control side effects of therapy will be given if patients have documented fevers and chills at any administration of peptide. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg p.o. every 4 hours). If patients require systemic steroid therapy, they will be taken off treatment.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

5.1.1 Research Bloods

- Leukapheresis:

  3-5L leukapheresis within 1 month prior to first vaccination and at 1 month after receiving 6 vaccines and at 7 months after receiving 6 vaccines if an immunologic response is noted at 1 month after receiving 6 vaccines. Collections at 1 and 7 months after receiving 6 vaccines may be delayed up to 12 weeks pending results of immune response testing.

- Peripheral sampling:
  - 30 mL of blood in purple top (CBC) tubes at baseline to establish autologous EBV-transformed lymphoblastoid cell lines for recognition studies.
  - At baseline and at the evaluations 1 month after receiving 3 vaccines and 6 vaccines a total of 50 mL of blood will be collected to isolate PBL for T cell subsets and CD4+ CD25+ (T regulatory cell) analysis, PBL will be cryopreserved and sera will be frozen.
    - 20 mL in 2 (10 mL) red top tubes
    - 30 mL in 3 (10 mL) purple top tubes.
    - Blood will be transported to the Thoracic Epigenetics Lab on ice and specimens will be tracked per Thoracic Epigenetics Lab SOP using the Labmatrix database.
  - Samples will be cryopreserved for bulk analysis.
- All specimens for each patient will be analyzed concurrently following the evaluation 1 month after receiving 6 vaccinations and the evaluation that follows 6 months later for responders.
- Serum and PBMC will be assessed with attention to serologic and cell mediated responses (as determined by ELISAs) to a standard panel of CT antigens and stem cell markers relative to those expressed in epigenetically modified and untreated autologous tumor cells or EBV-transformed B cells. Particular attention will be directed toward assessing responses to MAGE-A3 and NY-ES0-1.

5.1.2 Generation of Autologous EBV-Transformed B Cell Lines

Peripheral blood lymphocytes will be isolated by Ficoll gradient techniques and cultured in RPMI-10% human AB serum in the presence of supernatant from EBV-transformed B95 cells. Resulting lymphoblastoid clones will be expanded and cryopreserved for future use. HLA expression in autologous tumor cells and EBV-transformed B cells will be confirmed by PCR techniques in the NIH HLA lab.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.2.1 Thoracic Epigenetics Laboratory

This study will be conducted within the Thoracic Surgery Branch, NCI. Samples will be stored in designated monitored freezers (at least -20°C). All samples obtained on this study will be tracked using Labmatrix. Samples will be identified and tracked using unique identifiers linked to each subject's unique patient number (study number). Codes linking personal identifiable information to the unique identifier will be stored in secure, computer servers with limited coded access or locked file cabinets in the Thoracic Surgery Branch, NCI with access limited to the PI or study coordinator.

5.2.2 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues.

If the participant withdraws consent, his/her data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

Following completion of this study, if the subject has co-enrolled on 06C0014, samples will be transferred to the Tissue Procurement protocol and remain in storage in the Blood Processing Core (BPC) as detailed within 06C0014. If the subject has not co-enrolled on the Tissue Procurement protocol, the samples will be destroyed.

The PI will report any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into the in-house password protected electronic systems (C3D and Labmatrix), and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All Adverse Events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the time of first study intervention through at least 30 days after the last intervention. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

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

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

6.1.1 Exclusions to Routine Data Collection

The following events will be captured only in the source documents and will not be recorded in C3D:

- All Grade 1 events
- Grade 2 events that are not related to the treatment
- Grade 1, 2, and 3 Lab values drawn outside of the protocol specified time points that are not assessed as clinically significant by the PI or his designee and are not associated with an adverse event
- Events related to vascular access devices (occlusion, thrombi, hospitalizations for insertion or removal)

6.1.1.1 Concomitant Medications/Measures

All concomitant medications and measures will be captured in the source documents. Only those medications that the patient is taking at baseline on a routine basis or medications that cause an AE will be captured in C3D (e.g., onetime medications, PRN medications, supportive medications, electrolyte replacement and medications given to treat adverse events will not be captured in C3D).

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center).
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository, clinicaltrials.gov.
- BTRIS (automatic for activities in the Clinical Center).
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

This study research is not subject to the NIH genomic data sharing policy.

6.3 RESPONSE CRITERIA

Response to therapy will be assessed on the basis of molecular endpoints (i.e., immune response to CT antigens) as patients enrolled on this trial will have no evidence of disease at baseline. Specifically, response to vaccine will be the appearance of new serologic reactivity, or increase in existing antibody response to CT-X antigens, evidenced by IgM/IgG class switch or antibody titer. Additional indicators of response to therapy will be in vitro cell-mediated recognition of autologous tumor cells, or autologous EBV transformed lymphocytes exposed to sequential DAC/DP.

Approximately one month following the first six vaccinations, and every 6 months during retreatment, immunologic response to vaccinations will be assessed relative to baseline using the following parameters:

- ***Serologic responses to purified proteins:*** ELISA techniques will be used to examine antibody titers to a standardized panel of CT antigens and stem cell proteins expressed in thoracic malignancies. This analysis will be performed by Dr. Sacha Gnjatic, Mt. Sinai School of Medicine utilizing techniques that he developed and validated [120]. Coded human pre and post-treatment serum samples collected in association with this protocol will be transferred under Material Transfer Agreement (MTA) #35744-13 to Dr. Sacha Gnjatic, Ph.D., Tisch Cancer Institute, Mount Sinai School of Medicine, 1470 Madison Avenue, 5th Floor, Box 1128 New York NY 10029. As will be stipulated in the MTA, Dr. Gnjatic will perform ELISA of serologic responses to a panel of cancer-testis antigens including NY-ESO-1, MAGE, SSX and others utilizing techniques that he developed and validated [120]. Samples will be shipped overnight in dry ice via Fedex. Data generated by Dr. Gnjatic under these studies will be transferred to Dr. David S. Schrump for Dr. Schrump's use. Any of the following criteria will constitute immunologic response to vaccination:
    - Appearance of serologic reactivity against CT antigens in previously sero-negative patients
    - IgM→IgG class switch against CT antigens
    - Two fold or greater increase in titer of IgGs recognizing CT antigens induced in epigenetically-modified autologous tumor cells
- ***Analysis of pre and post-vaccine T-cell subsets including CD4+CD25+ FoxP3 T reg cells by flow cytometry techniques*:** Additional analysis of T reg function by in vitro assays may be undertaken if warranted, based on results of flow cytometry experiments.
- ***Cell Mediated Responses to CT-X Antigens in Autologous Tumor Cells:*** Cell mediated responses to CT-X antigens will be assessed using several in-vitro assays

selected on the basis of the HLA status of the patient, availability of autologous tumor cell lines or autologous tumor digests, or EBV transformed cell lines. If feasible, PBL from leukapheresis samples will be co-cultured with autologous tumor cell lines exposed to normal media or sequential Decitabine/Depsipeptide (DAC/DP). If autologous tumor lines (established under protocol 06C0014) are not available, autologous tumor cell digests may be used as targets. Additional recognition experiments will utilize autologous EBV-transformed B cells cultured in normal media (NM) or sequential DAC/DP as targets. For each of these experiments, tetanus toxoid will serve as a positive control. Recognition will be assessed by IFN-γ release assays. The immunologic response in these assays will be considered positive if at least a two-fold increase in vaccine-specific IFN-γ secretion is seen between the post-vaccination samples compared to the pre-vaccination specimens, and is reproducible in two sets of independent experiments. If two consecutive independent experiments do not provide the same answer, two additional independent experiments will be performed. If two or more of these four experiments are negative, then the patient will be considered a non-responder. These experiments will allow preliminary examination of immune response to CT-X antigens without constraints of patient HLA status, and defined CT-X antigens and epitopes. Additional assays may include the Elispot and tetramer assays, as well as experiments using transfection of specific CT-X genes encoding antigens recognized following vaccinations in individual patients.

It may take up to 12 weeks for these results to be available.

6.4 TOXICITY CRITERIA

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

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

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

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

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

7.2.2 IRB Requirements for PI Reporting at Continuing Review

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

7.3 NCI Clinical Director Reporting

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

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

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

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (weekly) when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2).

8.1.2 Serious Adverse Event (SAE)

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

- Death
- A life-threatening adverse event (see Section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization

- A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for subject convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

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

8.1.3 Life-Threatening

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

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

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

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

8.2 Assessment of Safety Events

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

SAEs will be:

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

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported.

8.3 Reporting of Serious Adverse Events

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

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

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

8.4 Reporting Pregnancy

8.4.1 Maternal Exposure

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

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

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

8.4.2 Paternal Exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of H1299 Lysate Vaccine.

Pregnancy of the participant's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

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

9 CLINICAL MONITORING

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

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

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

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

10 STATISTICAL CONSIDERATIONS

The primary objectives of this trial are to assess the frequency of immunologic responses to CTAs in patients with thoracic malignancies following vaccinations with H1299 cell lysate/Iscomatrix™ vaccines alone or with metronomic oral cyclophosphamide and celecoxib and to ascertain if metronomic oral cyclophosphamide and celecoxib enhances the frequency and spectrum of immune responses to CTAs in thoracic oncology patients receiving H1299 cell lysate/Iscomatrix™ vaccines.

The principal secondary objective of this trial is to examine if oral metronomic cyclophosphamide and celecoxib therapy primarily diminishes the percentage (and secondarily the number) of T regulatory cells and diminishes activity of these cells in patients with thoracic malignancies who are at risk of recurrence. We will also examine if H1299 cell lysate/ Iscomatrix™ vaccination enhances immunologic response to autologous tumor or epigenetically modified autologous EBV-transformed lymphocytes (B cells).

Following the principals of a Phase 2.5 design [121], the trial will randomize 28 patients per arm (56 total) to either receive vaccine alone or vaccine plus chemotherapy in order to have 80% power

to determine if the frequency of immune responses on the combination arm exceeds that of the vaccine alone arm, if the expected frequencies of immune responses on the two arms were 20% and 50%, using a one-sided 0.10 alpha level Fisher's exact test.

Patients on the trial will be stratified for thoracic vs. non-thoracic primary disease. Based on current referral patterns, it is anticipated that approximately 60-75% of the patients with primary thoracic neoplasms will have lung cancers, whereas the majority of patients with non-thoracic primary tumors will have sarcomas, with a minor number having adrenal-cortical carcinomas.

In addition to a comparison of the immune response rates on the two arms, the immune response rates on the individual arms will also be determined and reported with 95% confidence intervals.

In order to ensure that the trial does not continue to enroll patients in the event that evidence of immune response is not present on either arm, an early stopping rule will also be applied to each arm individually. Although there are various criteria for establishing whether an immune response has been identified, if no patients in the first 12 evaluable for immunological response on a given arm develop a clear immune response by any of the means allowed in this protocol, then no further patients will be enrolled on this arm as soon as this has been determined. This is because the upper one-sided 90% confidence interval bound on 0/12 is 17.5%. Thus, failure to find evidence of immune response in 12 patients on an arm would tend to make it unlikely that the true immune response rate is 20% or higher, which would be considered to be the threshold of what may be worthy of further development. Furthermore, if the true immune response rate were 20%, the probability of observing 0/12 immune responses is 6.9%, and the probability is between 0.2 and 3.2% if the true immune response rate were from 25 to 40%. This confirms that it is very unlikely to not have any immune responses in 12 patients if the true response rate were higher than 20%. As this is intended to provide a measure of safety in the event that the vaccine or combination is not minimally effective in producing an immune response, it will be desirable to pause accrual shortly after 12 patients have been enrolled on a given arm if this will be necessary to make this determination with certainty. In order to not disrupt the referral pattern to this study, up to 3 additional patients (15 total) may be enrolled on a given arm, if necessary in order to allow time to decide if results obtained from the first 12 warrant further accrual or would indicate that it be stopped as soon as the lack of immune response in these first 12 patients is determined. Should accrual end to the vaccine alone arm, accrual may still continue to the combination arm, and if accrual ends to the combination arm, accrual is permitted on the vaccine alone arm, provided that there is at least one response in the first 12 patients on that arm.

This primary objective will be addressed by performing analyses which investigate immunologic responses to a panel of CT antigens in vaccinated patients. An immunologic response will be any response of the type as described in Section **6.2** of the protocol. As this is an initial study seeking to determine in a somewhat broadly defined group of patients with thoracic malignancies if obtaining any evidence of an immune response is possible in modest numbers of subjects, immune responses determined via any accepted method described will be of interest. The actual number of such responses noted will be identified, and described according to type of response found, the types of cancers the responses were identified in, and other important characteristics of the response. All other evaluations using this endpoint will be done in an entirely exploratory manner, as necessary to describe the results obtained.

In order to determine if oral metronomic CP and celecoxib therapy diminishes the percentage of T regulatory cells on each arm, each of these parameters will be obtained at baseline and at the

conclusion of treatment. The difference, or the relative difference, in the values at the two time points will be obtained, and tested to determine if the difference is equal to zero. If a paired t-test is able to be used, with at least 20 evaluable patients, there is 81% power to detect a change equal to ¾ of a standard deviation of the change at the two-sided 0.025 significance level; this will be done in order to allow for a conservative adjustment due to determining the significance of the change in the percent of Tregs on two arms. In practice, a Wilcoxon signed rank test may be used if the differences, or the relative differences from baseline, are not normally distributed. We will then compare the frequency (and if possible magnitude) of immune responses in patients receiving vaccines with metronomic chemotherapy relative to those receiving vaccines alone.

For each patient in whom an immune response is detected, we will attempt to correlate T regulatory cell percentage and T regulatory function if possible using co-culture experiments using epigenetically modified autologous tumor or HLA matched tumor cells as targets. Because of the variability of the responses to be determined, and unknown reproducibility of some assays, these experiments will be considered as an entirely exploratory analysis.

It is expected that two to four patients per month may be enrolled onto this study. Thus, accrual of 60 subjects (56 for scientific purposes, but allowing for some individuals who may not be evaluable following initiation of vaccines) may be completed in approximately 2.5 years.

11 COLLABORATIVE AGREEMENTS

11.1 MATERIALS-COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (M-CRADA)

M-CRADA # 02584 between the Thoracic Surgery Branch, National Cancer Institute, and CSL Limited is in place. CSL Limited will supply the ISCOMATRIX adjuvant used in the study.

12 HUMAN SUBJECTS PROTECTION

12.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered on this protocol have a history of thoracic neoplasms, but have NED or MRD following therapy. Many of these individuals will experience recurrence and succumb to their diseases. This protocol is designed to evaluate the immunologic response, if any, to a vaccine regimen in patients without disease or minimal residual disease, as recent studies suggest that vaccines targeting CT-X antigens induce immunity more efficiently in patients with minimal or no disease relative to individuals with excessive tumor burdens [49, 52]. The experimental treatment has a chance to provide clinical benefit, although this is unknown. Subjects of both genders and all racial/ethnic groups are eligible. Efforts will be made to extend accrual to a representative population, but a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand, and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with gender or ethnic identity are noted, accrual may be expanded, or a follow-up study may be written to investigate these differences more fully.

12.2 PARTICIPATION OF CHILDREN

Because of the attendant risks associated with initial Pilot trials and because the administration of vaccines in combination with CP and celecoxib has unknown effects in children, patients less than 18 years of age will be excluded from this study until more data are available.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **12.5.1** for consent procedure.

12.4 EVALUATION OF POTENTIAL BENEFITS AND RISKS/DISCOMFORTS

The risks and benefits of participation for adults who become unable to consent on study are no different than those described for the rest of the study population.

12.4.1 Risks

The risks to the patient participating in this trial are anticipated to be small, and are primarily the risks associated with preparation and administration of the vaccines. These potential risks and benefits will be carefully discussed with each patient at the time consent is obtained.

12.4.1.1 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.4.1.2 Urine Collection

There is no physical risk involved with urine collection.

12.4.1.3 Fecal Occult Blood Test

There is no physical risk involved with fecal collection for this test.

12.4.1.4 Electrocardiogram (EKG)

No risks are associated with this procedure. The patient may experience minimal discomfort during the procedure.

12.4.1.5 Leukapheresis

The risks of leukapheresis include pain, bruising, lightheadedness or dizziness, nausea, vomiting and chills. Bruising may last up to 72 hours.

During the leukapheresis procedure, platelet counts may decrease because platelets are collected with the white blood cells.

Tingling around the mouth, fingers, or toes and mild muscle cramps may develop from slight lowering of the blood calcium by the blood thinner used during the procedure. These symptoms can be treated by either temporarily stopping the procedure or by giving a calcium pill. Leukapheresis uses a completely closed sterile system. The risk of infection is minimized by cleaning the skin before the needle stick. No infections from leukapheresis have been noted in thousands of such procedures performed over the last 10 years at the NIH.

Rarely, there can be a malfunction of the apheresis machinery that might prevent the return of your blood being processed in the machine. The amount of blood lost would be very small and not harmful. It is also rare for people to faint, have seizures, or have air trapped in the bloodstream.

Temporary or permanent nerve damage may occur at the needle placement sites. This is very rare. At the NIH, to this point, there have been no cases of permanent nerve damage with leukapheresis.

12.4.2 Benefits

There may be some direct benefit to patients who participate in this trial since it is anticipated that the vaccine regimen may enhance antitumor immunity that could eradicate micro metastases, thereby preventing or delaying disease recurrence. The greatest benefit will be the information regarding the feasibility, toxicities, and immunologic responses to H1299 lysate/ ISCOMATRIX™ vaccines relative to CT-X protein expression in the respective primary tumors.

12.5 CONSENT PROCESSES AND DOCUMENTATION

The informed consent document will be provided to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with local policy, including Policy M20-1) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigators, funding agencies, the Investigational New Drug (IND) sponsor and regulatory authorities, as applicable. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 H1299 LYSATE VACCINE PREPARATION

The H1299 cell line was established at the NCI from a patient with NSCLC. This cell line broadly expresses CT-X antigens at high levels due to stable amplification of the X chromosome. A Master Cell Bank (MCB) was produced at NCI/Frederick from H1299 stocks obtained from ATCC, and subsequently transferred to the Thoracic Epigenetics Laboratory, Thoracic Surgery Branch, NCI.

Cryopreserved H1299 cells from the MCB will be thawed, and cultured in RPMI, with 10% fetal bovine serum (FBS) and penicillin/streptomycin (pen/strep) in T-75 flasks. Quantitative RT-PCR techniques will be used to assess CT gene expression in the cultured cells. Following confirmation of sterility, purity, identity, and potency, cells will be enzymatically dissociated, washed extensively in 0.9% Sodium Chloride, then lysed by freeze-thaw technique followed by centrifugation to remove cellular debris according to the SOP. Lysates from $1x10^8$ cells (8-10 mg protein) in 0.5-1 mL PBS will be aliquoted and frozen at -80°C. On the day of vaccination, a lysate vial will be thawed, and the lysate mixed with 1 mL ISCOMATRIX™ adjuvant diluted to 180 ISCO units in 0.9% Sodium Chloride. Briefly, lysates will be drawn up in a 2 mL luer lock syringe with spin-lock connectors and the syringe will be connected to a 3-way stopcock. ISCOMATRIX™ adjuvant will be drawn in a separate syringe and connected to the stopcock.

Contents of the syringes will be passed back and forth between 2 syringes at least 10 times. The tumor cell lysate/ISCOMATRIX™ mixture will then be drawn into two 1 mL syringes, placed on ice, and transferred to Thoracic Surgery Personnel in the Clinic for administration via deep SQ injection into the proximal thigh within 2 hours after preparation.

14.2 ISCOMATRIX™ ADJUVANT

14.2.1 How supplied

ISCOMATRIX™ adjuvant is prepared by combining, under controlled conditions, solutions of ISCOPREP™ saponin (fractionated from an extract of the plant material of the *Quillaja saponaria* tree) with Dipalmitoyl-sn-phosphatidylcholine (DPPC)and cholesterol, in a detergent (MEGA 10), to solubilize the lipids, and buffered isotonic saline (pH 6.2). The adjuvant cage-like structures are formed during ultrafiltration to remove the detergent, and then sterilized by filtration. This adjuvant will be supplied by CSL Behring, Inc. in bulk at approximately 4000 ISCO units/ml, and will be aliquoted by Clinical Center Pharmacy staff in 1 ml aliquots, and stored at 2-8° C undiluted, until use. On the day of vaccination, the ISCOMATRIX™ adjuvant will be diluted in 0.9% Sodium Chloride to yield 180 ISCO units/ml.

14.2.2 Administration

Within 2 hours before vaccination, 1 ml of the diluted ISCOMATRIX™ adjuvant will be mixed together with 0.5- 1.0 ml of the H1299 lysates through luer lock syringes, attached to a 3-way stopcock and administered via deep SQ injection into the thigh.

14.2.3 Toxicities

The current formulation of ISCOMATRIX ™ adjuvant has been administered to nearly 1800 individuals with HIV or HCV, healthy volunteers, and cancer patients. Toxicity reports from the 1582 patients enrolled on completed studies of the current formulation include no vaccine related deaths and one possibly related SAE of cardiac arrest in a patient with underlying cardiac disease. The most common injection site reactions reported in the manufacturer's studies (N=576) were pain (70.8%), swelling (33.7%) and redness (33.9%). Systemic reactions included myalgia (45.1%), fatigue (32.3%) and headache (31.0%) comprising a transient flu-like syndrome. No significant changes in systemic markers of auto-immunity (anti-cardiolipin antibodies, anti-β2-glycoprotein-1 antibodies, or anti-nuclear antibodies), allergy (IgE levels), or inflammation (C Reactive Protein) have been observed [80, 81].

14.2.4 Drug Interactions

None known

14.3 CYCLOPHOSPHAMIDE

(Please see package insert for more detailed information)

2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2-oxazaphosphorine 2-oxide monohydrate. Synonyms: Cytoxan®, CTX, CPM

14.3.1 Source

Cyclophosphamide tablets will be purchased commercially and supplied by the NIH Clinical Center Pharmacy in 25 mg and 50 mg strengths. However, in the rare event when a patient loses his/her prescription and needs immediate resupply, a finite number of pills can be prescribed by

the patient's private oncologist to allow continuation of treatment while a new supply is being sent from the NIH CC pharmacy.

14.3.2 Storage and stability

Tablets must be stored at or below 77 degrees Fahrenheit (25 degrees Celsius) away from moisture and direct sunlight

14.3.3 Dosage and administration

Patients should take the medications at approximately the same time each day (± 6 hours) as described in Section **3.1.2**. There is no information that suggests food will alter the bioavailability of this drug.

14.3.4 Toxicities

The most common toxicities associated with cyclophosphamide include nausea and vomiting, alopecia as well as leucopenia and these are related to dosing levels. In the previous clinical trials of metronomic CPM therapy, the rates of nausea/vomiting and alopecia were extremely low. In addition hemorrhagic cystitis and secondary malignancies have been seen with CPM. For a complete listing of toxicities associated with CPM, refer to the CPM package insert

14.4 CELECOXIB

(Please see Package Insert for more detailed information)

Synonyms: Celebrex®

14.4.1 Description and How Supplied

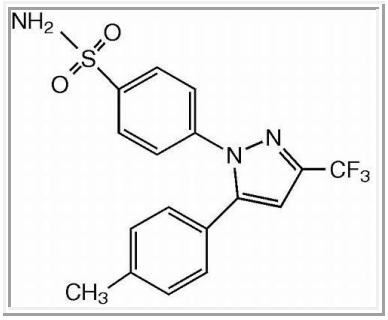
Celecoxib is a non-steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. Celecoxib will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and supplied for use in this study as 400 mg capsules. However, in the rare event when a patient loses his/her prescription and needs immediate resupply, a finite number of pills can be prescribed by the patient's private oncologist to allow continuation of treatment while a new supply is being sent from the NIH CC pharmacy.

14.4.2 Mode of Action

The mechanism of action of celecoxib is believed to be due to inhibition of prostaglandin synthesis, primarily via inhibition of cyclooxygenase-2 (COX-2), and at therapeutic concentrations in humans, celecoxib does not inhibit the cyclooxygenase-1 (COX-1) isoenzyme. In animal colon tumor models, celecoxib reduced the incidence and multiplicity of tumors.

14.4.3 Chemical Identification

Celecoxib is chemically designated as 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide and is a diaryl-substituted pyrazole. It has the following chemical structure:



The empirical formula for celecoxib is $C_{17}H_{14}F_3N_3O_2S$, and the molecular weight is 381.38.

14.4.4 Formulation

Celecoxib is commercially available. Celecoxib oral capsules contain 100 mg, 200 mg or 400 mg of celecoxib.

The inactive ingredients in celecoxib capsules include: croscarmellose sodium, edible inks, gelatin, lactose monohydrate,

14.4.5 Administration

Celecoxib should be taken orally with food.

14.4.6 Toxicities

COMMON

abdominal pain, diarrhea, dyspepsia, flatulence, nausea, back pain, peripheral edema, dizziness, headache, insomnia, pharyngitis, rhinitis, sinusitis, URI, rash

SERIOUS (incidence)

melena (<2%), GI bleeding (<0.1%), GI perforation (<0.1%), allergic reactions (<2%), liver function test abnormalities (<2%), hepatitis, jaundice (<0.1%), aggravated hypertension (<2%), arrhythmias (<0.1%), CHF (<0.1%), MI (<0.1%), anemia (<2%), acute renal failure (<0.1%), interstitial nephritis (<0.1%), erythema multiforme (rare), exfoliative dermatitis (rare), Stevens Johnson syndrome (rare), toxic epidermal necrolysis (rare)

BOX WARNING

Cardiovascular Risk

- Celecoxib may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. All non-steroidal anti-inflammatory drugs (NSAIDs) may have a similar risk. This risk may increase with duration of use. Patients with cardiovascular disease or risk factors for cardiovascular disease may be at greater risk.
- celecoxib is contraindicated for the treatment of perioperative pain in the setting of coronary artery bypass graft (CABG) surgery.

Gastrointestinal Risk

- NSAIDs, including celecoxib, cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients are at greater risk for serious gastrointestinal events.

- Drug Interactions (partial list)
    - aspirin
    - fluconazole
    - lithium
    - warfarin
    - ACE-inhibitors and Angiotensin II Antagonists
    - furosemide
    - methotrexate
    - concomitant NSAID use

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16 APPENDICES

16.1 APPENDIX 1: SUPPLEMENTARY TABLES

Supplementary Table A: Cancer-Testis Genes and Their Chromosomal Locations

Gene family	Family members	CT identifier	Chromosomal localization
MAGEA	MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA5, MAGEA6, MAGEA8, MAGEA9, MAGEA10, MAGEA11, MAGEA12	CT1	Xq28
BAGE	BAGE	CT2	21p11.1
MAGEB	MAGEB1, MAGEB2, MAGEB3, MAGEB4, MAGEB5, MAGEB6	CT3	Xp21.3
GAGE	GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GAGE7, GAGE8	CT4	Xp11.23
SSX	SSX1, SSX2, SSX3, SSX4	CT5	Xp11.23-p11.22.
NY-ESO-1	CTAG1B, CTAG2	CT6	Xq28
MAGEC1	MAGEC1	CT7	Xq26
SYCP1	SYCP1	CT8	1p12-p13
BRDT	BRDT	CT9	1p22.1
MAGEC2	MAGEC2	CT10	Xq27
SPANX	SPANXA1, SPANXB1, SPANXC, SPANXD	CT11	Xq27.1
XAGE	XAGE1	CT12	Xp11.22
HAGE	DDX43	CT13	6q12-q13.
SAGE	SAGE1	CT14	Xq26.
ADAM2	ADAM2	CT15	8p11.2.
PAGE-5	PAGE5	CT16.1	Xp11.23
LIPI	LIPI	CT17	21q11.2
NA88A pseudogene	VENTXP1	CT18	Xp21.3.
IL13RA	IL13RA2	CT19	Xq13.1-q28
TSP50	TSP50	CT20	3p12–14
CTAGE-1	CTAGE1	CT21.1	18p11.2
SPA17	SPA17	CT22	11q24.2
ACRBP	ACRBP	CT23	12p12-p13.
CSAG	CSAG1, CSAG2	CT24	Xq28.
MMA1	DSCR8	CT25	21q22.2.

Gene family	Family members	CT identifier	Chromosomal localization
CAGE/DDX53	DDX53	CT26	Xp22.11
BORIS	CTCFL	CT27	20q13.31
HOM-TES-85	LUZP4	CT28	Xq23
AF15q14	CASC5	CT29	15q14
HCA661	TFDP3	CT30	Xq26.2
JARID1B	JARID1B	CT31	1q32.1.
LDHC	LDHC	CT32	11p15.3-p15.5
MORC	MORC1	CT33	3q13
SGY-1	DKKL1	CT34	19q13.33
SPO11	SPO11	CT35	20q13.2-q13.3
TPX1	CRISP2	CT36	6p21-qter
NY-SAR-35	FMR1NB	CT37	Xq27.3-q28.
FTHL17	FTHL17	CT38	Xp21
NXF2	NXF2	CT39	Xq22.1
TAF7L	TAF7L	CT40	Xq22.1
TDRD1	TDRD1	CT41	10q25.3.
TEX15	TEX15	CT42	8p12
FATE	FATE1	CT43	Xq28
TPTE	TPTE	CT44	21p11
CT45	CT45-1, CT45-2, CT45-3, CT45-4, CT45-5, CT45-6	CT45	Xq26.3
HORMAD1	HORMAD1	CT46	1q21.2.
CT47	CT47-1, CT47-2, CT47-3, CT47-4, CT47-5, CT47-6, CT47-7, CT47-8, CT47-9, CT47-10, CT47-11, CT47-12	CT47	Xq24
SLCO6A1	SLCO6A1	CT48	5q21.1
TAG	TAG	CT49	Na
LEMD1	LEMD1	CT50	1q32.1
HSPB9	HSPB9	CT51	1p36.2-p35
CCDC110	CCDC110	CT52	4q35.1
ZNF165	ZNF165	CT53	6p21.3
SPACA3	SPACA3	CT54	17q11.2
CXorf48	CXorf48	CT55	Xq26.3

Gene family	Family members	CT identifier	Chromosomal localization
THEG	THEG	CT56	19pter-p13
ACTL8	ACTL8	CT57	1p36.2-p35
NLRP4	NLRP4	CT58	1p36.2-p35
COX6B2	COX6B2	CT59	19q13.42
LOC348120	LOC348120	CT60	15q11.2
CCDC33	CCDC33	CT61	15q24.1
LOC196993	LOC196993	CT62	15q23
PASD1	PASD1	CT63	Xq28
CT64	CT64/BX103208	CT64	3q26.1.
TULP2	TULP2	CT65	19q13.1.
CT66	CT66/AA884595	CT66	7q11.22.
KLKBL4	KLKBL4	CT67	16q21
RBM46	RBM46	CT68	4q32.1
CT69	CT69/BC040308	CT69	6q23.2
CT70	CT70/BI818097	CT70	Na
SPINLW1	SPINLW1	CT71	20q12-q13.2
TSSK6	TSSK6	CT72	19p13.11
ADAM29	ADAM29	CT73	4q34.
CCDC36	CCDC36	CT74	3p21.31
LOC440934	LOC440934	CT75	2q36.1
SYCE1	SYCE1	CT76	10q26.3
CPXCR1	CPXCR1	CT77	Xq21.3
TSPY1	TSPY1	CT78	Yp11.2
TSGA10	TSGA10	CT79	2q11.2.
PIWIL2	PIWIL2	CT80	8p21.3
ARMC3	ARMC3	CT81	10p12.31
AKAP3	AKAP3	CT82	12p13.3.
CXorf61	Cxorf61	CT83	Xq23
PBK	PBK	CT84	8p21.2
C21orf99	C21orf99	CT85	21q11.2
OIP5	OIP5	CT86	15q15.1
CEP290	CEP290	CT87	12q21.32

Gene family	Family members	CT identifier	Chromosomal localization
CABYR	CABYR	CT88	18q11.2
SPAG9	SPAG9	CT89	17q21.33.
MPHOSPH1	MPHOSPH1	CT90	10q23.31
ROPN1	ROPN1	CT91	3q21.1.
PLAC1	PLAC1	CT92	Xq26
CALR3	CALR3	CT93	19p13.1
PRM	PRM1/PRM2	CT94.1	16p13.2
CAGE1	CAGE1	CT95	6p24.3
CT96	TTK	CT96	6q13-q21
LY6K	LY6K	CT97	8q24.3
IMP-3	IMP-3	CT98	7p11.
AKAP4	AKAP4	CT99	Xp11.2
DPPA2	DPPA2	CT100	3q13.13
KIAA0100/MLAA-22	KIAA0100	CT 101	7q11.2
TCC52	TCC52	CT102	9p13.3
SEMG1	SEMG1	CT103	20q12-q13.2
POTE	POTE21, POTE2, POTE8, POTE14, POTE15, POTE18, POTE22	CT104	2q21.1
FLJ36144/MAD-CT2	MAD-CT2	CT105	15q11.2.
NUF2/CDCA1	CDCA1	CT106	1q23
RHOXF2/PEPP2	PEPP2	CT107	Xq24
OTOA	OTOA	CT108	16p12.2
CCDC62	CCDC62	CT109	12q24.31
GPATCH2	GPATCH2	CT110	1q41

Na: not available

Supplementary Table B: Frequency of CT Gene Expression in Various Human Cancers as Determined by Semi-Quantitative RT-PCR

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/ Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
MAGEA1/CT1.1	22	-	18	2	53	29	28	80	0	49	48	28	-	15	0	14
BAGE1/CT2.1	15	-	10	0	-	-	8	-	0	4	26	15	-	0	0	6
MAGEB1/CT3.1	0	0	17	0	-	0	0	-	0	14	22	-	-	0	0	9
GAGE/CT4.1	12	-	9	0	-	-	19	38[b]	1	19	28	31	-	10	0	25
SSX2/CT5.2	44	6	7	12	-	-	35	9[b]	36	16	35	-	-	40	5	50
NY-ESO-1/CT6.1	80	0	30	0	-	0	-	29	0	17	34	25	0	25	9	0
MAGEC1/CT7.1	44	-	30	10	-	-	36	-	-	33	70	-	-	-	-	60
SYCP1/CT8	-	47	20	0	-	7	-	28[b]	0	7	14	0	-	0	8	0
BRDT/CT9	0	-	0	0	8	-	8	-	-	25	0	-	-	-	0	-
MAGEE1/CT10	44	-	38	0	-	-	36	-	-	24	50	-	-	-	-	0
SPANXC/CT11.3	9	-	25	22	0	-	-	-	-	33	70	-	0	-	-	-
XAGE-1a/CT12.1a	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	22
HAGE/CT13	24	37	5	31	27	-	-	20	9	32	17	-	-	22	6	20
SAGE/CT14	12	0	5	0	20	-	17	-	4	22	4	-	-	0	5	5

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/ Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
ADAM2/CT15	-	-	0	0	-	-	-	-	-	0	0	0	-	-	12	-
PAGE-5/CT16	-	-	5	11	-	-	-	-	-	39	22	0	-	-	44	-
LIP1/CT17	-	-	5	0	-	-	-	-	-	0	0	0	-	-	25	-
NA88/CT18	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-
TSP50/CT20	-	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-
CTAGE-1/CT21.1	-	-	-	-	-	-	-	-	35	-	-	-	-	-	-	-
SPA17/CT22	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-
OYTES1/CT23	28	-	40	15	-	0	-	40	-	20	-	-	-	-	0	-
MMA1a/CT25.1a	-	-	0	0	0	-	-	-	-	40	26	-	0	-	-	18
CAGE/CT26	-	-	-	-	-	89	-	-	-	100	-	-	-	-	-	-
HOMTES85/CT28	-	35	0	10	-	-	-	19	-	28	36	32	-	0	-	-
D40/CT29	-	20	-	13	-	0	-	-	-	41	-	36	27	-	-	-
HCA661/CT30	0	-	-	-	-	0	0	29	-	-	20	-	-	-	-	-
PLU-1/CT31	-	-	86	-	-	-	-	-	-	-	-	-	-	-	-	-
LDHC/CT32	-	-	35	15	-	-	-	-	-	47	44	42	-	37	57	-
MORC/CT33	-	-	0	0	-	-	-	-	-	18	18	14	-	0	0	-

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/ Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
SGY-1/CT34	-	-	20	0	-	-	-	-	-	12	25	57	-	12	0	-
SPO11/CT35	-	-	0	0	-	-	-	-	-	0	6	0	-	0	0	-
TPX1/CT36	-	-	15	0	-	-	-	-	-	-	6	14	-	37	14	-
NYSAR35/CT37	42	-	23	0	8	-	-	-	-	17	6	8	-	-	0	8
FTHL17/CT38	22	-	14	0	0	-	10	-	0	25	0	-	-	0	0	0
NXF2/CT39	19	-	0	11	12	-	5	-	0	15	55	-	-	14	0	27
TAF7L/CT40	10	-	0	0	0	-	10	-	0	9	21	-	-	0	0	12
TDRD1/CT41.1	28	-	37	0	10	-	22	-	5	5	0	-	-	38	0	0
TEX15/CT42	21	-	0	0	20	-	11	-	0	21	27	-	-	12	33	28
FATE/CT43	-	-	-	21	-	7	-	66	-	0	-	-	-	-	-	-
TPTE/CT44	-	-	-	0	-	0	-	39	-	36	-	-	-	-	-	-

[a]Abbreviations: Blad, bladder; Brn, brain; Brst, breast; Col, colon; Gas, gastric; H/N, head and neck; Leuk, leukemia; Lymph, lymphoma, NSCLC, non-small cell lung carcinoma; Mel, melanoma; Ov, ovarian; Pancr, pancreatic; Pros, prostate; Sarc, sarcoma.

Reprinted from ref [122]

16.2 APPENDIX 2: H1299 CELLS

ATCC

Product Sheet

NCI-H1299 (ATCC® CRL-5803™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: NCI-H1299 (ATCC® CRL-5803™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: lung; derived from metastatic site: lymph node
Disease: carcinoma; non-small cell lung cancer
Age: 43 years adult
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin:X
CSF1PO:12
D13S317:12
D16S539:12,13
D5S818:11
D7S820:10
THO1:6,9.3
TPOX:8
vWA: 16,17,18

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete growth medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

ATCC

Product Sheet

NCI-H1299 (ATCC® CRL-5803™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: NCI-H1299 (ATCC® CRL-5803™)

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Email: Tech@atcc.org

Or contact your local distributor

2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes are given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Cryopreservation Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The cells have a homozygous partial deletion of the p53 protein, and lack expression of p53 protein. They reported to be able to synthesize the peptide neuromedin B (NMB) at 0.1 pmol/mg protein, but not the gastrin releasing peptide (GRP).

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

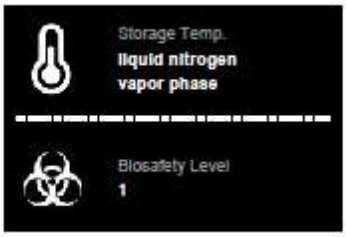
This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort

ATCC

Product Sheet

NCI-H1299 (ATCC® CRL-5803™)

Please read this FIRST



Intended Use

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The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: NCI-H1299 (ATCC® CRL-5803™)

is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.
Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
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Or contact your local distributor

16.3 APPENDIX 3: 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.

16.4 Appendix 4: Sample Medication Self-Administration Record and Patient Diary

NAME **DATE** **April 2013**

Sun	Mon	Tue	Wed	Thu	Fri	Sat
	1	2 labs/vaccine	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

Take Medications: celecoxib 400mg 2x/day EVERYDAY, Cyclophosphamide 50 mg 2x/day ALL Red days ONLY. Both medications will be taken 2x/day with food. Vaccinations will be given on the BLUE days in clinic - plan to stay in clinic 1 hour post injection.

GI	Dates	Treated at Home	Treated in doctor's office	Hospitalized	Comments
diarrhea					
nausea					
vomiting					
mouth sores					
neuropathy					
tingling hands and feet					
sensitivity to cold					
general					
Fever					
Infection					
swelling arms/legs					
fatigue					
Injection site					
redness/swelling					
pain					

shortness of breath					
bruising/bumps					
swollen glands near injection site					
rash					
Respiratory					
sore throat					
runny nose					
cough					
Other:					

16.5 APPENDIX 5: DOSE MODIFICATION TABLE

Adverse Event (CTCAE criteria)	Dose Modification
Neutrophil count decreased ≥ Grade 3 Platelet count decreased ≥ Grade 3 Lymphocyte count decreased ≥ Grade 3	• Reduce cyclophosphamide to next lower dose level • Follow CBC and platelets biweekly • If neutropenia, lymphocyte count decreased and/or thrombocytopenia have: o resolved to ≤ Grade 2, continue cyclophosphamide at same dose level. o not resolved to ≤ Grade 2, hold cyclophosphamide and continue to follow CBC and platelets biweekly. • When neutropenia, lymphocyte count decreased and/or thrombocytopenia have resolved to ≤ Grade 2, continue cyclophosphamide at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue cyclophosphamide
Nausea/Vomiting ≥ Grade 3	• Hold celecoxib • Follow weekly • When the toxicity resolves to ≤ Grade 2, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Abdominal pain ≥ Grade 3	• Hold celecoxib • Follow weekly • When the toxicity resolves to ≤ Grade 2, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Electrolyte imbalances ≥ Grade 3	• Hold celecoxib • Follow twice a week • When the toxicity resolves to ≤ Grade 1, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Dyspepsia ≥ Grade 2	• Hold celecoxib • Follow weekly • When the toxicity resolves to ≤ Grade 1, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Alanine aminotransferase increased ≥ Grade 2 Aspartate aminotransferase increased ≥ Grade 2	• Hold celecoxib • Follow weekly • When the toxicity resolves to ≤ Grade 1, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Creatinine ≥ Grade 2	• Hold celecoxib

	• Follow weekly • When the toxicity resolves to ≤ Grade 1, restart at next lower dose level. • If AE occurs at the last allowable dose reduction, discontinue celecoxib
Gastrointestinal hemorrhage ≥ Grade 2	• Discontinue celecoxib

16.6 APPENDIX 6: STUDY CALENDAR/TRIAL SCHEMA

16.6.1 Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Plus Chemo Cohort

Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Plus Chemo Cohort

			Course 1											
Procedure	**Prior to Vaccination**		**Cycles 1**				**Cycle 2**				**Cycle 3**			
	wk -4	wk -1	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
Celecoxib		X	X	X	X	X	X	X	X	X	X	X	X	X
Cyclophosphamide		X		X		X		X		X		X		X
H1299 Vaccine			X				X				X			
H&P, Toxicity Assessment		X					X				X			
Lipid Panel[5], Thyroid Panel[5]	X													
Labs[1]		X	X	X	X	X	X		X		X		X	
Research Bloods[2]		X L												X
			Course 2											
Procedure			**Cycles 4**				**Cycle 5**				**Cycle 6**			
			wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
Celecoxib			X	X	X	X	X	X	X	X	X	X	X	X
Cyclophosphamide				X		X		X		X		X		X
H1299 Vaccine			X				X				X			
H&P, Toxicity Assessment			X				X				X			
Labs[1]			X				X				X			
Research Bloods[2]														X L[4]
Immune Response Evaluation[3]														X

Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Plus Chemo Cohort													
		Course 3											
Procedure		**Cycle 7**				**Cycle 8**				**Cycle 9**			
		wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
Celecoxib		X	X	X	X	X	X	X	X	X	X	X	X
Cyclophosphamide			X		X		X		X		X		X
H1299 Vaccine										X			
H&P, Toxicity Assessment		X				X				X			
Labs[1]		X				X				X			
Research Bloods													X
		Course 4											
Procedure		**Cycle 10**				**Cycle 11**				**Cycle 12**			
		wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
Celecoxib		X	X	X	X	X	X	X	X	X	X	X	X
Cyclophosphamide			X		X		X		X		X		X
H1299 Vaccine										X			
H&P, Toxicity Assessment		X				X				X			
Labs[1]		X				X				X			
Research Bloods[2]													X L[4]
Immune Response Evaluation[3]													X

[1] See Section **3.5** for detailed information.

[2] L = Leukapheresis; See Section **5.1.1** for detailed information.

[3] See Section **6.2** for detailed information.

[4] Only performed if response noted at the evaluation ~1 month after receiving 6 vaccines.

[5] Lipid Panel=total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol-calculated; Thyroid Panel=TSH, free T4.

16.6.2 Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Cohort

Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Cohort														
			Course 1											
Procedure	**Prior to Vaccination**		**Cycles 1**				**Cycle 2**				**Cycle 3**			
	wk -4	wk -1	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
H1299 Vaccine			x				x				x			
H&P, Toxicity Assessment		x					x				x			
Lipid panel[5], Thyroid Panel[5]	x													
Labs[1]		x	x	x	x	x	x		x		x		x	
Research Bloods[2]		X L												x
			Course 2											
			Cycles 4				Cycle 5				Cycle 6			
			wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
H1299 Vaccine			x				x				x			
H&P, Toxicity Assessment			x				x				x			
Labs[1]			x				x				x			
Research Bloods														X L[4]
Immune Response Evaluation[3]														X
			Course 3											
Procedure			**Cycle 7**				**Cycle 8**				**Cycle 9**			
			wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
H1299 Vaccine											x			
H&P, Toxicity Assessment			x				x				x			
Labs[1]			x				x				x			
Research Bloods[2]														x

Trial Schema: H1299 lysate/ISCOMATRIX™ Vaccine Cohort													
		Course 4											
Procedure		**Cycle 10**				**Cycle 11**				**Cycle 12**			
		wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4	wk 1	wk 2	wk 3	wk 4
H1299 Vaccine										x			
H&P, Toxicity Assessment		x				x				x			
Labs[1]		x				x				x			
Research Bloods[2]													X L[4]
Immune Response Evaluation[3]													X

[1] See Section **3.5** for detailed information.

[2] L = Leukapheresis; See Section **5.1.1** for detailed information.

[3] See Section **6.2** for detailed information.

[4] Only performed if response noted at the evaluation ~1 month after receiving 6 vaccines.

[5] Lipid Panel=total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol-calculated; Thyroid Panel=TSH, free T4.