	RATIONALE: Drugs used in chemotherapy use different ways to stop tumor cells from dividing so they stop growing or die. Vaccines may make the body build an immune response to kill tumor cells. Sargramostim may stimulate a person's immune system and help to kill tumor cells. PURPOSE: Phase II trial to study the effectiveness of vaccine therapy plus sargramostim following chemotherapy in treating patients who have stage III or stage IV non-Hodgkin's lymphoma. Detailed Description: OBJECTIVES: I. Determine the ability of recombinant idiotype immunotherapy to stimulate a specific immune response against the B cell idiotype of the malignant clone that constitutes the tumor in patients with previously untreated stage III or IV indolent non-Hodgkin's lymphoma. II. Determine the safety and toxicity of this treatment regimen using Genitope Corporation's molecular rescue technology in this patient population. OUTLINE: Patients receive induction chemotherapy consisting of oral cyclophosphamide, vincristine, and prednisone (CVP). Treatment repeats every 3 weeks until the maximal clinical response is achieved followed by 2 additional courses of consolidation therapy for up to a maximum of 10 courses. Patients not achieving adequate response receive up to 6 courses of alternate chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine, and prednisone. At 3 months or up to 1 year following completion of chemotherapy, patients achieving adequate disease response receive vaccination consisting of recombinant tumor derived immunoglobulin idiotype with keyhole limpet hemocyanin conjugate subcutaneously (SQ) at 2 sites immediately followed by sargramostim (GM-CSF) SQ on day 1. Patients receive GM-CSF alone on days 2-4. Vaccination repeats every 4 weeks for 4 doses, followed 12 weeks later by the fifth and final dose. Patients are followed every 3 months for 2 years, every 6 months for 2 years, and then annually thereafter until disease progression. PROJECTED ACCRUAL: Not specified
Active, not recruiting	Human Telomerase Reverse Transcriptase Messenger RNA (hTERT mRNA) Transfected Dendritic Cell Vaccines Condition: Metastatic Prostate Cancer 2010–2011 Intervention: Biological: hTERT mRNA DC

	The purpose of this research is to develop a new and powerful type of immune therapy for prostate cancer patients. This therapy involves vaccinations with special stimulator cells found in the human body called dendritic cells. These dendritic cells can take up proteins released from cancer cells and present pieces of these proteins to immune cells called T lymphocytes to create a strong stimulatory signal to fight the cancer. One of these proteins is called telomerase, which is found on prostate cancers and is critically important for prostate cancer cells to grow. However, in most cancer patients, the immune system does not adequately destroy the tumor because the T cells are not stimulated sufficiently. T cells require strong stimulation before they grow and become active against cancer cells. We have discovered that substances called ribonucleic acids (RNA), which carry the genetic instructions for the production of telomerase, can be used to overcome this problem and stimulate a strong immune response in cancer patients. In order to test this hypothesis we have designed a clinical study and will enroll patients with**metastatic prostate cancer expressing telomerase** in order to determine whether or not this vaccine will stimulate T cells, which can recognize and kill prostate tumor cells. The main objectives of this study are to find out whether injections with dendritic cells grown from blood cells and "pulsed" (mixed together for a short period of time) with RNA derived from the patient's own tumor are: 1.Safe without inducing any major side effects. 2.And effective in boosting the patient body's immunity against telomerase expressing prostate cancer cells. 3.Finally, we will test whether or not tumor shrinkage based on serum PSA levels or on X-ray studies will occur. We hope that this new form of immune therapy, although in its infancy, will ultimately slow down tumor growth and prolong survival of prostate cancer patients. **ARM1: Biological: hTERT mRNA DC.** Subjects receive 1x107 cells per infusion administered ID at study week 1, 2, 3, 4, 5, 6 then receive**5x106 cells per infusion administered ID at study weeks 10, 14, 18, 22, 26, 30, 34, 38, 42, 46 and 50.** **ARM2: Biological: hTERT mRNA DC.** Subjects receive 1x107 cells per infusion administered ID at study week 1, 2, 3, 4, 5, 6 then receive**1x107 cells per infusion administered ID at study weeks 10, 14, 18, 22, 26, 30, 34, 38, 42, 46 and 50.**

	Primary objectives of trial include to evaluate the safety and biologic efficacy of hTERT mRNA transfected dendritic cells (DC), applied in a prime-boost format, to stimulate **hTERT-specific CD4+ and CD8+ T-cell responses** in subjects with metastatic prostate cancer. Secondary objectives include estimating objective clinical response, the duration of such responses, **progression-free survival and overall survival** among all subjects. The hTERT mRNA-transfected DC vaccine platform has previously been studied in several phase I/II trials and has demonstrated safety and bioactivity in subjects with metastatic prostate and renal cell carcinomas. The objective of this trial is to enhance the observed bioactivity of the vaccine by using a prime-boost strategy. This is an open label, uncontrolled safety and efficacy study. Subjects with metastatic prostate cancer will be eligible for this study and will receive 1x107 cells administered ID at study week 1,2,3,4,5, and 6 (Prime). Thereafter, subjects will be randomized with equal probability to receive either 5x106 cells administered ID at study week 10 followed by monthly immunizations (Treatment arm A) or 1x107 cells administered ID at study week 10 followed by monthly immunizations (Treatment arm B). The safety, biologic and clinical efficacy of each regimen will be analyzed. The study will be solely conducted at the University of Florida in Gainesville, FL. Subjects will be recruited through the oncology clinics of the Departments of Urology and Radiation Oncology. The vaccine will be manufactured in a dedicated GMP-compliant cell production facility located on the 4th floor of the Cancer Genetics Research Institute. Immunological testing will be performed in the Immunological Monitoring Core laboratory of the Department of Urology using standardized assay systems. Subjects with histologically or clinically confirmed**metastatic prostate cancer (stages pT1-4, N0-3, M+)** are eligible for this study. **Subjects treated with medical hormone ablative therapy (LHRH analogues or estrogens**) should continue to receive LHRH analogues only. In subjects receiving nonsteroidal medical hormonal treatment (i.e. flutamide or bicalutamide) and who are experiencing a rising PSA, a 4 week period of observation will be required following the discontinuation of the nonsteroidal antiandrogen prior to study entry. Subjects will be excluded from study if they have received chemotherapy or other forms of immunotherapy in the 4 weeks prior to study entry. **They must not have a history of autoimmune disease,** serious intercurrent chronic or acute illness, pulmonary disease, active hepatitis, serologic evidence for HIV, or be receiving corticosteroid or immunosuppressive therapy. All subjects must be older than 18 years. This is a randomized phase II clinical trial, in which up to 36 subjects will be randomized with equal probability to one of the two treatment arms. The objective of this trial is to decide which of the two treatment regimens should be selected for further testing. Hence, the primary objective of this trial is to select the arm with the highest biologic response and the first ranked arm will be selected for further study in a larger efficacy trial.
Completed	Vaccine Therapy Plus Sargramostim Following Chemotherapy in Treating Patients With Previously Untreated Aggressive Non-
	Condition: Lymphoma 2000-2010
	Interventions: Biological: keyhole limpet hemocyanin; Biological: sargramostim; Biological: tumor cell-based vaccine therapy; Drug: cyclophosphamide; Drug: doxorubicin hydrochloride; Drug: mitoxantrone hydrochloride; Drug: prednisone; Drug: vincristine sulfate

	RATIONALE: Drugs used in chemotherapy use different ways to stop cancer cells from dividing so they stop growing or die. Vaccines may make the body build an immune response to kill cancer cells. Colony-stimulating factors such as sargramostim may increase the number of immune cells found in bone marrow or peripheral blood and may help a person's immune system recover from the side effects of chemotherapy. PURPOSE: Phase II trial of vaccine therapy plus sargramostim following chemotherapy in treating patients who have previously untreated aggressive non-Hodgkin's lymphoma. OBJECTIVES: I. Determine the ability of recombinant idiotype immunotherapy to stimulate a specific immune response against the B cell idiotype of the malignant clone that constitutes the tumor in patients with previously untreated aggressive non-Hodgkin's lymphoma. II. Determine the safety and toxicity of this treatment regimen using Genitope Corporation's molecular rescue technology in this patient population. OUTLINE: Patients receive induction chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or cyclophosphamide, mitoxantrone, vincristine, and prednisone (CNOP). Treatment repeats every 3 weeks until the maximal clinical response is achieved followed by 2 additional courses of consolidation therapy for up to a maximum of 6 courses. At 2-6 months following completion of chemotherapy, patients achieving adequate disease response receive vaccination consisting of recombinant tumor derived immunoglobulin idiotype with keyhole limpet hemocyanin conjugate subcutaneously (SQ) followed by sargramostim (GM-CSF) SQ, each at 2 separate sites on day 1. Patients receive GM-CSF alone on days 2-4. Vaccination repeats every 4 weeks for 4 doses, followed 3 months later by the fifth and final dose. Patients are followed every 3 months for 2 years, every 6 months for 2 years, and then annually thereafter until disease progression. PROJECTED ACCRUAL: Not specified
Active, not recruiting	Vaccine Therapy in Treating Patients With Primary Stage II Melanoma Condition: Melanoma (Skin) 2000–2009 Interventions: Biological: GM2–KLH vaccine; Biological: QS21; Procedure: adjuvant therapy
	Adjuvant Ganglioside **GM2-KLH/QS-21 V**accination: Post-Operative Adjuvant Ganglioside GM2-KLH/QS-21 (BMS-248479) Vaccination Treatment After Resection of Primary Cutaneous Melanoma Thicker Than 1.5mm (AJCC/UICC Stage II, T3-T4N0M0), a 2-Arm Multicenter Randomized Phase III Trial vs. Observation. OBJECTIVES: •Compare the effect of immunization with GM2-KLH and QS21 to observation on the **disease-free survival** of patients with primary cutaneous stage II melanoma after adequate surgery. •Determine overall survival and toxicity in the two treatment arms. OUTLINE: This is a randomized, open-label, parallel, multicenter study. Patients are stratified according to participating center, tumor thickness (**greater than 1.5 to 3.0 mm vs greater than 3.0 to 4.0 mm vs greater than 4.0 mm**), gender, ulceration (yes vs no), and presence of additional staging procedures of regional lymph nodes (yes vs no). Patients are randomized to one of two arms. •Arm I: Patients are vaccinated with **GM2-KLH and QS21 subcutaneously on day 1 of weeks 1-4, 12, 24, 36, 48, 60, 72, 84, 96, 120, and 144 for a total of 14 vaccinations.** •Arm II: Patients undergo observation. Patients are followed every **6 months for 7 years.** PROJECTED ACCRUAL: A total of 1300 patients (650 per arm) will be accrued for this study within 36 months.

Active, not recruiting	Vaccine Therapy and Interleukin-12 With or Without Interleukin-2 in Treating Patients With Metastatic Melanoma Condition: Melanoma (Skin) 2005-2011 Interventions: Biological: MART-1 antigen; Biological: NA17-A antigen; Biological: aldesleukin; Biological: gp100 antigen; Biological: recombinant MAGE-3.1 antigen; Biological: recombinant interleukin-12; Biological: therapeutic autologous lymphocytes	MAGE-3/Melan-A/gp100/NA PBMC, rhIL-12 MAGE-3/Melan-A/gp100/NA17 Peptide-pulsed autologous PBMC, rhIL-12 with IL-2 MAGE-3/Melan-A/gp100/NA17 Peptide-
	Purpose of investigation: Primary hypotheses: Immunization of patients with 4 melanoma antigen peptides will induce augmented specific IFN-y-producing CD8+ T cells against all 4 antigens simultaneously. Immunization with 4 melanoma antigen peptides will increase the response rate from 10% to 30%. Administration of low-dose IL-2 following each vaccine will result in a greater than 3-fold increase in specific T cells compared to no IL-2. Secondary hypotheses: Immunization will clear the blood of detectable circulating melanoma cells. Tumors that grow despite induction of melanoma antigen-specific T cells may lack expression of antigens, class I MHC, or the TAP peptide transporter, or may fail to show increased expression of mRNA for IFN-y or perforin. Tumors that resist vaccination may express a different array of genes than those that are susceptible to vaccination. **Primary:** •The primary hypothesis is immunization of patients with 4 melanoma antigen peptides will induce augmented **specific IFN-.-producing CD8+ T cells against all 4 antigens** simultaneously, and to determine the clinical response rate. Based on the above preclinical and Phase I results, a logical strategy for a second generation melanoma vaccine has emerged. A randomized Phase II study in metastatic melanoma patients will be undertaken. Patients first will be HLA-typed; **HLA-A2-positive** patients will be eligible for screening. When feasible, each patient will undergo a tumor biopsy to screen for expression of MAGE-3, Melan-A, gpIOO, and NAI 7 using RT-PCR and immunohistochemistry, to determine whether T cells are present in the lesion, to measure cytokine gene expression by RT-PCR, and to perform gene array analysis. In addition, blood cells will be analyzed for certain parameters of T cell function. Patients will be randomized to cohorts A (no IL-2) or B (with low-dose IL-2). For treatment, peripheral blood will be collected and fractionated by density centrifugation to isolate PBMC as a source of APC. The PBMC will be divided into four pools, each of which will be incubated with one of the following peptides: MAGE-3, Melan-A, gp 100, or Ni 7A. The peptide-loaded cells will then be washed and recombined into a single suspension in PBS, and lethally irradiated. Approximately 120 x 106 pulsed cells will be injected subcutaneously at a site near a lymph node not thought to be involved with tumor. The subcutaneous route has been selected for the reasons of safety, efficacy in the preclinical model, and the goal of targeting the vaccine to a draining lymph node. rhIL-12 (4 .tg straight dose) will then be given subcutaneously adjacent to the vaccine site days 1,3, and 5 of each cycle. This dose and schedule was found to be effective in our phase I study. In one-half of the patients (cohort B), IL-2 (I MU straight dose) will be administered subcutaneously daily, days 7-18. Re-immunization along with rhIL-12 followed by IL-2 (if assigned) will be performed at 3 week intervals as in cycle I.	

	On day 1 of each cycle, peripheral blood will be collected to measure peptide-specific IFN-y production. Before treatment and after every 3 cycles, PBMC will be collected to quantify peptide specific CD8 T cells by flow cytometric analysis with peptide/HLA-A2 tetramers, and evidence for a molecular response will be assessed by performing RT-PCR. for melanoma antigens on peripheral blood samples. In addition, prior to treatment, after the first 3 cycles, and at the time of going off- study, a tumor biopsy will be performed to assess the immune response in the tumor microenvironment, including gene array analysis. It is hoped that these studies will uncover the reason for lack of clinical response in patients with residual tumors. Clinical response will be assessed as a secondary outcome.
Completed	Monoclonal Antibody A1G4 Plus BCG in Treating Patients With Cancer Conditions: Neuroblastoma; Sarcoma 1999–2010 Interventions: Biological: BCG vaccine; Biological: monoclonal antibody A1G4 anti-idiotype vaccine
	Monoclonal antibodies can locate tumor cells and either kill them or deliver tumor-killing substances to them without harming normal cells. Combining monoclonal antibody A1G4 with BCG may kill more tumor cells. PURPOSE: Phase I trial to study the effectiveness of monoclonal antibody A1G4 plus BCG in treating patients with cancer OBJECTIVES: •Assess the toxicity and feasibility of immunizing patients with anti-idiotypic rat monoclonal antibody A1G4 combined with Bacillus Calmette Guerin (BCG) adjuvant. •Determine whether immunization with A1G4 combined with BCG results in an immune response directed against GD2 ganglioside in patients. OUTLINE: All patients are treated with A1G4 diluted in sterile physiologic saline mixed with Bacillus Calmette Guerin (BCG) organisms. The vaccine is injected intradermally in multiple sites. Booster immunizations are administered during weeks 2, 4, 8, 12, 20, 28, 36, 44, 52. Immunizations are not administered in limbs where draining lymph nodes have been surgically removed or previously irradiated. Isoniazid is administered for 5 days after each BCG injection. If severe skin reactions are present at the injection site, the BCG dose is decreased. If skin reactions persist, the BCG dose is stopped but A1G4 injections continue. At least 6 patients are accrued at each dose level of A1G4. Dose escalation is not carried out until patients have been followed for at least 8 weeks after the first immunization without encountering grade 3 or worse non-skin toxicity. If 0-1 patient experiences dose limiting toxicity (DLT) at a given dose level, then patients are accrued to the next higher dose level. If 2 or more patients experience DLT, the MTD is defined as the previous dose level. Patients are followed for at least 1 year. PROJECTED ACCRUAL: A total of 24 patients are expected to complete this study. If patients are removed early from the study prior to evaluation for serological response, additional patients will be accrued until 6 patients are evaluable for serological response.
Active, not recruiting	Vaccine Therapy With or Without Imiquimod in Treating Patients Who Have Undergone Surgery for Stage II, Stage III, or Stage Condition: Melanoma (Skin) 2005–2009

	Interventions:	Biological: incomplete Freund's adjuvant; Biological: multi-epitope melanoma peptide vaccine; Biological: sargramostim; Biological: tetanus toxoid helper peptide; Drug: dimethyl sulfoxide; Drug: imiquimod; Procedure: adjuvant therapy	
	Primary: •Safety if less than 33% of patients experience a dose-limiting at day 22 [Designated as safety issue: Yes] **Secondary** •Immune response by Elispot assay at day 22 [Designated as safety issue: No] **OJECTIVES: 1)** termine the safety of adjuvant transdermal vaccine therapy comprising **multi-epitope melanoma peptides** (MP), **tetanus toxoid helper peptide** (TET), and **GM-CSF** in combination with **Montanide ISA-51** or dimethyl sulfoxide with or without imiquimod in patients who have undergone surgical resection for stage II-IV melanoma. 2) etermine, preliminarily, the immunogenicity of these regimens in these patients. 3) orrelate, preliminarily, transdermal administration of these vaccines with the recruitment and maturation of epidermal Langerhans cells in these patients. 4) etermine, preliminarily, the effects of timing of subsequent vaccine therapy comprising MP, TET, and GM-CSF emulsified in Montanide ISA-51, administered intradermally and subcutaneously, on the persistence of immune response in these patients. **OUTLINE:** This is a randomized, open-label study. Patients are randomized to 1 of 4 treatment arms. •**Arm I:** Patients receive vaccine therapy comprising multi-epitope melanoma peptides, tetanus toxoid helper peptide, and GM-CSF emulsified in Montanide ISA-51 transdermally (TD) on days **1, 8, and 15**. Patients then receive the vaccine intradermally (ID) and subcutaneously (SC) on days**29, 50, 71, 92, 113, and 134**. •**Arm II:** Patients receive vaccine therapy as in arm I. Patients also receive imiquimod topically on days 0, 7, and 14. •**Arm III:** Patients receive vaccine therapy comprising MP, TET, GM-CSF, and dimethyl sulfoxide TD on days 1, 8, and 15. Patients then receive vaccine therapy comprising MP, TET, and GM-CSF emulsified in Montanide ISA-51 ID and SC on days 29, 50, 71, 92, 113, and 134. •**Arm IV:** Patients receive vaccine therapy as in arm III and imiquimod as in arm II. In all arms, treatment continues in the absence of disease progression or unacceptable toxicity. After completion of study treatment, patients are followed at 3 and 5 weeks and then at disease progression.		
Active, not recruiting	Vaccine Therapy With or Without Sargramostim in Treating Patients With Stage IIB, Stage IIC, Stage III, or Stage IV Melanoma		·Diagnosis of melanoma ◦Stage IIB, IIC, III, or IV disease ·Must express HLA-A1, -A2, or -A3
	Condition:	Melanoma (Skin) 2004-2009	
	Interventions:	Biological: incomplete Freund's adjuvant; Biological: multi-epitope melanoma peptide vaccine; Biological: sargramostim	
	OBJECTIVES: 1) Compare immune response in patients with stage IIB-IV melanoma treated with vaccination comprising multiple synthetic melanoma peptides and Montanide ISA-51 with vs without sargramostim (GM-CSF). 2) Compare immune response in patients treated with these vaccinations administered at 1 vs 2 sites. **OUTLINE:** This is a randomized, open-label study. Patients are randomized to 1 of 4 treatment arms. •Arm I: Patients receive vaccination comprising multiple synthetic melanoma peptides and Montanide ISA-51 at 1 injection site. •Arm II: Patients receive vaccination comprising multiple synthetic melanoma peptides and Montanide ISA-51 at 2 injection sites. •Arm III: Patients receive vaccination comprising multiple synthetic melanoma peptides, Montanide ISA-51, and sargramostim (GM-CSF) at 1 injection site. •Arm IV: Patients receive vaccination comprising multiple synthetic melanoma peptides, Montanide ISA-51, and GM-CSF at 2 injection sites. **In all arms, treatment repeats once weekly for 6 weeks**. Patients return for booster vaccinations at weeks **12, 26, 39, and 52.** PROJECTED ACCRUAL: A maximum of 124 patients will be accrued for this study.		
Active, not recruiting	Vaccine Therapy Plus Interleukin-2 With or Without Interferon Alfa-2b in Treating Patients With Stage III Melanoma		
	Condition:	Melanoma (Skin) 1999-2011	
	Interventions:	Biological: interleukin-2 liposome; Biological: polyvalent melanoma vaccine; Biological: recombinant interferon	

	Phase II Trial of the Effects of Interferon Alfa-2b on the Immunogenicity of a Polyvalent Melanoma Antigen Vaccine in Patients With Stage III Malignant Melanoma Detailed Description: OBJECTIVES: I. Determine the effect of interferon alfa-2b on the potentiation of antimelanoma antibodies and cellular immune responses induced by immunization to a polyvalent melanoma vaccine and interleukin-2 in patients with stage III malignant melanoma. II. Determine the optimal dose of interferon that will maximally stimulate these responses in these patients. III. Determine the toxicity of this regimen in these patients. OUTLINE: This is a randomized study. Patients are randomized into a vaccine treated control arm or to receive one of two doses of interferon alfa-2b plus vaccine. All patients receive polyvalent melanoma vaccine incorporated into interleukin-2 liposomes. The vaccine is administered intradermally every 2 weeks for 8 weeks, monthly for 3 months, and then every 3 months for a total of 2 years or until disease progression. Patients assigned to arms II or III also receive interferon alfa-2b subcutaneously, at one of two doses, three times a week for 2 years. Patients are followed for survival. PROJECTED ACCRUAL: A total of 32 patients will be accrued for this study within 18 months. Histologically proven, surgically resected stage III melanoma Clinically positive nodes AND/OR At least 2 histologically positive nodes**HLA-A2, A3, A11, or A26** positive Intact cellular immunity as evidenced by at least 5 mm reaction at 48 hours to at least 1 of the following recall antigens: PPD Mumps Candida Streptokinase streptodornase OR able to be sensitized to dinitrochlorobenzene
Completed	Vaccine Therapy in Treating Patients With Metastatic Melanoma of the Eye Conditions: Extraocular Extension Melanoma; Recurrent Intraocular Melanoma 2007 Drug: gp100 antigen; Drug: interleukin-2; Drug: MART-1 antigen; Drug: Montanide ISA-51
	Phase II Study of gp100:209-217 (210M) Antigen and MART-1:26-35 (27L) Antigen Emulsified in Montanide ISA-51 in Patients With Metastatic Ocular Melanoma Detailed Description: OBJECTIVES: I. Determine the clinical response in patients with metastatic ocular melanoma treated with gp100:209-217 (210M) antigen and MART-1:26-35 (27L) antigen emulsified in Montanide ISA-51. II. Determine the clinical benefit of interleukin-2 in combination with this vaccine in these patients. PROTOCOL OUTLINE: Patients receive vaccine subcutaneously once weekly. Treatment repeats every 4 weeks for a total of 6 courses in the absence of disease progression or unacceptable toxicity. Patients with progressive disease may receive vaccine SC on day 1 followed by interleukin-2 IV over 15 minutes every 8 hours for a maximum of 12 doses. Treatment repeats every 3 weeks for at least 4 courses in the absence of disease progression or unacceptable toxicity. PROJECTED ACCRUAL: A total of 15-25 patients will be accrued for this study within 1 year. Diagnosis of metastatic ocular melanoma Progressive disease Measurable disease HLA-A*201 positive --Prior/Concurrent Therapy-- Biologic therapy: At least 3 weeks since prior biologic therapy Chemotherapy: At least 3 weeks since prior chemotherapy Endocrine therapy: At least 3 weeks since prior endocrine therapy No concurrent steroid therapy Radiotherapy: At least 3 weeks since prior radiotherapy Surgery
Completed	Fludarabine Followed by Vaccine Therapy and White Blood Cell Infusions in Treating Patients With Unresectable or Metastatic Condition: Melanoma (Skin) 2004-2010 Interventions: Biological: gp100 antigen; Biological: incomplete Freund's adjuvant; Biological: keyhole limpet hemocyanin; Drug: fludarabine phosphate; Procedure: peripheral blood stem cell transplantation

	A Pilot Trial of Therapeutic Vaccination With a Modified gp100 Melanoma Peptide (gp100:209-217(210M)), Montanide ISA 51, and KLH With Reconstitution After Chemotherapy to Induce Lymphopenia in Patients With Metastatic Melanoma **Primary** 1) Toxicity by clinical and laboratory observation at 1 month. 2) Antigen-specific T-cell responses by tetramer analysis, ELISPOT, and cytokine flow cytometry periodically **Secondary**: 1) Compare 2 different dosing schedules of fludarabine in terms of lymphocyte recovery using a complete blood count periodically 2) Tumor regression by standard imaging at study completion **Primary 1)** etermine the toxicity and immune effects of vaccination comprising modified gp100 peptide (gp100:209-217[210M]), Montanide ISA-51, and keyhole limpet hemocyanin followed by peripheral blood mononuclear cell reinfusion after treatment-induced lymphopenia with fludarabine in patients with unresectable or metastatic melanoma. 2) Determine the induction of antigen-specific T-cell responses in patients treated with this regimen. 3) Determine the kinetics and duration of immune response in patients treated with this regimen. 4) Compare the immunologic effects of this regimen in these patients with historical results. **Secondary 1)** ompare 2 different dosing schedules of fludarabine, in terms of induction of lymphopenia and granulocytopenia and on the induction of a specific immune response to this vaccine, in these patients. **OUTLINE:** This is a pilot, randomized study. Patients are randomized to 1 of 2 treatment arms. Within 2 weeks before the start of fludarabine, all patients undergo leukapheresis over 4-6 hours for the collection of peripheral blood mononuclear cells (PBMCs). •**Arm I:** Patients receive fludarabine IV over 30 minutes on days 1-5. •**Arm II:** Patients receive fludarabine as in arm I on days 1, 3, and 5. In both arms, patients receive autologous PBMCs IV over approximately 30 minutes on day 8 and vaccination comprising gp100:209-217(210M) peptide, Montanide ISA-51, and keyhole limpet hemocyanin subcutaneously on days 8, 22, 36, 50, and 64. Patients with stable or responding disease continue to receive vaccination on day 78 and then every 28-31 days for up to 1 year. Patients are followed every 3 months. PROJECTED ACCRUAL: A total of 20 patients (10 per treatment arm) will be accrued for this study within 2 years.
Completed	Vaccine Therapy With or Without Interleukin-2 in Treating Patients With Metastatic Melanoma Conditions: Stage IV Melanoma; Recurrent Melanoma 2007 Interventions: Drug: gp100 antigen; Drug: interleukin-2 — gene therapy
	Phase II Study of DNA Encoding the gp100 Antigen Alone or in Combination With Interleukin-2 in Patients With Recurrent Metastatic Melanoma
Completed	Vaccine Therapy in Treating Patients With Metastatic Melanoma Who Are Undergoing Surgery for Lymph Node and Tumor Condition: Melanoma (Skin) 1999-2011 Interventions: Biological: aldesleukin; Biological: gp100 antigen; Biological: tyrosinase peptide

	A Phase I-II Trial of Antigen-Pulsed Autologous Dendritic Cells for Induction of Anti-Tumor Immunity in Patients Completing Lymphadenectomy for Metastatic Melanoma OBJECTIVES: I. Determine the safety and toxicity of intravenous injections of autologous cultured dendritic cells pulsed with either gp100 and tyrosinase peptides or autologous melanoma tumor cell lysates in patients with metastatic melanoma. II. Determine whether treatment with melanoma tumor antigen pulsed autologous dendritic cells results in increased in vitro tumor specific cytotoxic T-cell responses. III. Determine whether this treatment can induce positive skin test responses to tumor antigens. IV. Evaluate the disease free and overall survival of these patients. OUTLINE: This is a randomized, dose escalation study. Approximately 1-2 weeks following surgical lymphadenectomy, patients undergo leukapheresis to collect dendritic cells and are then divided into 3 groups. Group A consists of patients without adequate tumor for preparation of tumor lysate and who have tumors that express tyrosinase or gp100 with types HLA-A1, A2, or A3. Group B consists of the patients who have adequate tumor for lysate preparation but who do not type for HLA-A1, A2, or A3 (required for the peptide pulsed protocol). Group C are the patients with adequate tumor who are eligible for the peptide pulsed protocol. Group A patients receive autologous dendritic cells pulsed with appropriate peptide antigens. Group B patients are treated with autologous dendritic cells pulsed with autologous tumor cell lysates. Group C patients are randomized to receive dendritic cells pulsed with either peptide antigens or tumor lysate. All patients are administered intravenous active immunotherapy for 4 monthly intervals. The dose of the immunizations is escalated for each cohort of three patients that is accrued in each of the groups mentioned above. Each immunization at each dose level is followed by three days of interleukin-2 administered subcutaneously twice daily. Patients are followed at least 5 years for survival. PROJECTED ACCRUAL: There will be 100 patients accrued in this study over 2 years. There will be 50, 20, and 30 patients in groups A, B, and C, respectively. Histologically confirmed metastatic melanoma involving cervical, axillary, inguinal, groin, or iliac lymph nodes All gross disease is resected at the time of surgical lymphadenectomy No distant metastases	
Recruiting	Vaccine Therapy With or Without Cyclophosphamide in Treating Patients With Recurrent or Refractory Multiple Myeloma Condition: Multiple Myeloma and Plasma Cell Neoplasm 2007-2012 Interventions: Biological: oncolytic measles virus encoding thyroidal sodium iodide symporter; Drug: cyclophosphamide; Genetic: reverse transcriptase-polymerase chain reaction; Other: flow cytometry; Other: immunologic technique; Other: laboratory biomarker analysis; Procedure: biopsy	

	Phase I Trial of Systemic Administration of Edmonston Strain of Measles Virus, Genetically Engineered to Express NIS, With or Without Cyclophosphamide, in Patients With Recurrent or Refractory Multiple Myeloma **Primary: 1)** Toxicity. 2) Maximum tolerated dose [Designated as safety issue: Yes] **Secondary**: 1) Hematologic response (complete response, very good partial response, minimal response). 2) Viral replication and shedding. 3) Biodistribution and kinetics of viral spread and NIS gene expression. 4)Tolerability [Designated as safety issue: Yes] **Biological: MV-NIS** Dose escalation theme. Start at 10^7 TCID50 increase by a factor of 3 to a final dose of 81x10^7 TCID50. Other Name: oncolytic measles virus encoding thyroidal sodium iodide symporter. Other: I-123 prior MV-NIS 5 mCi Oral Any time pre-MV-NIS (for baseline I-123 scan) Other: I-123 post MV-NIS 5 mCi Oral at Days 3, 8, and 15 (two additional doses may be given for imaging based on imaging results) Other Name: 5 mCi Oral at Days 3, 8, and 15 (two additional doses may be given for imaging based on imaging results) Drug: Liothyronine 0.025 mg - 1 oral tablet three times daily. Starting 4 days prior to MV-NIS administration through day of last 123I scan (no longer than Day 29) Other Name: Cytomel **Biological: MV-NIS** **D**ose escalation theme. Start at 10^7 TCID50 increase by a factor of 3 to a final dose of 81x10^7 TCID50. Other Name: oncolytic measles virus encoding thyroidal sodium iodide symporter Drug: cyclophosphamide 10mg/kg Other: I-123 prior MV-NIS 5 mCi Oral Any time pre-MV-NIS (for baseline I-123 scan) Other: I-123 post MV-NIS 5 mCi Oral at Days 3, 8, and 15 (two additional doses may be given for imaging based on imaging results) Other Name: 5 mCi Oral at Days 3, 8, and 15 (two additional doses may be given for imaging based on imaging results) Drug: Liothyronine 0.025 mg - 1 oral tablet three times daily. Starting 4 days prior to MV-NIS administration through day of last 123I scan (no longer than Day 29) ther Name: Cytomel

	Primary: •Determine the safety and toxicity of Edmonston vaccine strain oncolytic measles virus encoding thyroidal sodium iodide symporter (MV-NIS) when administered with or without cyclophosphamide in patients with relapsed or refractory multiple myeloma. •Determine the maximum tolerated dose of MV-NIS when administered with or without cyclophosphamide in these patients. **Secondary:** •Determine the time course of viral gene expression and viral elimination, and the biodistribution of virally infected cells at various time points after treatment with these regimens using iodine I 123 gamma camera imaging. •Assess viral replication, viremia, viral shedding in urine and respiratory secretions, and viral persistence after treatment with these regimens. •Monitor humoral responses to MV-NIS in these patients. •Explore the antimyeloma efficacy (i.e., clinical response rate, time to progression, progression-free survival, duration of response) of the virus using standard myeloma response criteria as well as immunoglobulin free light chain measurements. OUTLINE: This is a dose-escalation study of oncolytic measles virus encoding thyroidal sodium iodide symporter (MV-NIS). Patients are stratified according to receipt of cyclophosphamide during study treatment (yes vs no). Patients are initially accrued to part 1. Once the maximum tolerated dose (MTD) of MV-NIS alone is determined, subsequent patients are accrued to part 2. •Part 1 (MV-NIS alone : Patients receive MV-NIS IV over 30 minutes on day 1. Cohorts of 3-6 patients receive escalating doses of MV-NIS until the MTD is determined. The MTD is defined as the dose preceding that at which 2 of 6 patients experience dose-limiting toxicity. •Part 2 (MV-NIS and cyclophosphamide): Patients receive cyclophosphamide IV over 30 minutes on day -1 and MV-NIS IV over 30 minutes on day 1. Cohorts of 3-6 patients receive escalating doses of MV-NIS* in combination with cyclophosphamide until the MTD is determined. The MTD of MV-NIS is defined as in part 1. NOTE: *Starting dose of MV-NIS is the MTD determined in part 1. Blood and bone marrow samples are obtained for research studies, including flow cytometry, at baseline and at week 6. Serial measurements of viral RNA in mononuclear cells are conducted in samples of blood, saliva, and urine on days 3, 8, and 15 and are tested for viral replication by quantitative reverse transcriptase-polymerase chain reaction. Measles virus-specific immunity is evaluated at baseline and on day 42. After the completion of study treatment, patients are followed periodically for 1 year.PROJECTED CCRUAL: A total of 54 patients will be accrued for this study.
Recruiting	Safety Study of Cancer Specific Epitope Peptides Cocktail and Cyclophosphamide for Advanced or Relapsed Solid Tumors Condition: Metastatic Solid Tumors　2008　九大 Intervention: Biological: 5 peptide vaccines of KOC1, TTK, CO16, DEPDC1, MPHOSPH1

	Phase I Study of Tumor Specific Potentiated Vaccine Therapy Using Cyclophosphamide Combined Epitope Peptide Cocktail for Progressive/Relapsed Solid Tumors(GI/Lung/Cervical Cancer) **Primary:** •safety of the cyclophosphamide combined tumor specific epitope peptide cocktail [Time Frame: 2 years] [Designated as safety issue: Yes] **Secondary**: •immunological efficacies and clinical efficacies of the cyclophosphamide combined tumor specific epitope peptides cocktail [Time Frame: 2.5 years] [KOC1, TTK, CO16(URLC10), DEPDC1, MPHOSPH1 have been identified using genome-wide expression profile analysis by the use of cDNA microarray in the previous studies. The investigators have determined the HLA-A*2402 restricted epitope peptides respectively derived from KOC1, TTK, CO16(URLC10), DEPDC1, and MPHOSPH1 showed strong INF-gamma production when stimulated with the appropriate targets expressing the appropriate protein and HLA-A*2402. Furthermore, when vaccinated these peptides, specific CTLs were determined after the vaccination. Therefore the investigators focused on the prevention of further expansion of the solid tumors highly expressing these 5 proteins using these 5 peptides.
Completed	Carcinoembryonic Antigen-loaded Dendritic Cells in Advanced Colorectal Cancer Patients Conditions: Colorectal Cancer; Liver Metastases 2005-2010 Intervention: Biological: CEA-loaded dendritic cell vaccine
	Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. As such they are currently used in clinical vaccination protocols in cancer patients. We evaluate the ability of mature DCs pulsed with carcinoembryonic antigen (CEA)-peptide (arm A) or electroporated with CEA-mRNA (arm B) to induce CEA-specific T cell responses in patients with resectable liver metastases from colorectal cancer. To evaluate immune responses, CEA-specific T cell reactivity is monitored in peripheral blood, resected abdominal lymph nodes, tumor tissue and biopsies of vaccination sites and post-treatment DTH skin tests. Patients are vaccinated intradermally and intravenously with CEA-peptide pulsed mature DCs three times prior to resection of liver metastases. In 2007 a side-study has been added (arm C), in which patients with stage III or high-risk stage II colorectal cancer that are amenable for standard adjuvant oxaliplatin/capecitabine therapy are vaccinated with CEApeptide-pulsed DCs. Also in this group, safety and immune responses in peripheral blood and the DTH-skin test are the primary endpoints. Results are compared with the results obtained in arm A. Biological: CEA-loaded dendritic cell vaccine: Carcinoembryonic antigen (either peptide or mRNA) loaded dendritic cells. Biological: CEA-loaded dendritic cell vaccine: Carcinoembryonic antigen (either peptide or mRNA) loaded dendritic cells. Biological: CEA-loaded dendritic cell vaccine: Carcinoembryonic antigen (either peptide or mRNA) loaded dendritic cells. Primary: •immunological response against carcinoembryonic antigen and the control protein KLH. •Toxicity
Completed	Evaluate the Immunogenicity & Safety of GSK Biologicals' HPV Vaccine in Female Subjects Aged 10-14 Years Conditions: Human Papillomavirus (HPV) Infection; Cervical Neoplasia Intervention: Biological: HPV-16/18 L1/AS04
Completed	Vaccine Therapy Plus GM-CSF in Treating Patients With Multiple Myeloma Undergoing Bone Marrow or Peripheral Stem Cell

Condition:	Multiple Myeloma and Plasma Cell Neoplasm
Interventions:	Biological: keyhole limpet hemocyanin; Biological: sargramostim

Phase I Trial of Post Transplant Immunization With Autologous Myeloma Idiotype-KLH/GM-CSF In Myeloma Patients Following Autologous or Allogeneic Marrow or Stem Cell Transplantation

Primary: •Toxicities graded using the National Cancer Institute (NCI) Common Toxicity Criteria [Time Frame: Up to 2 years]

Descriptive statistics will be used to summarize changes from baseline in clinical laboratory parameters for each cohort.

•Immune response [Up to 2 years] Descriptive statistics will be used to summarize changes from baseline in clinical laboratory parameters for each cohort.

PRIMARY OBJECTIVES:

I. To determine the safety of multiple subcutaneous vaccinations with myeloma Id-KLH (idiotype-keyhole limpet hemocyanin) with GM-CSF (sargramostim) in post allogeneic transplant myeloma patients, or with GM-CSF +/- interleukin (IL)-2 (aldesleukin) in post autologous transplant myeloma patients.

II. To evaluate patients pre and post bone marrow transplantation (BMT) for evidence of endogenous idiotype specific immune response.

III. To characterize the time course, specificity and persistence of antibody and T cell immune response to myeloma idiotype and to KLH induced by myeloma Ig (Id) immunization.

IV. To clone, expand and characterize T cells specific for the tumor idiotype. V. Monitor myeloma involvement in bone marrow and serum paraprotein level following vaccination.

VI. Use stored patient samples to clone, expand, and characterize T cells specific for myeloma antigens other than idiotype and identify the antigens they recognize so that they can be used in future studies.

OUTLINE: Patients receive autologous immunoglobulin idiotype-KLH conjugate vaccine combined with sargramostim subcutaneously (SC) in weeks 0, 2, 6, and 10 and sargramostim SC once daily (QD) for three days following each vaccine injection. Some patients also receive aldesleukin SC daily from weeks 2-14.

After completion of study treatment, patients are followed up every 3 months for 1 year and then every 6 months for 1 year.

資料２　案２

治療用がんワクチンの評価における考慮事項に関するガイドライン（案）

目次（2013.8.23 改訂案）

資料２　案２

治療用がんワクチンの臨床評価等における考慮事項に関するガイドライン（素案）

1.はじめに

本ガイドラインはがんワクチンの臨床試験の開始に当たっての留意点について考察したものである。また、がんワクチンはがん細胞に特異的に発現するがん抗原に対する特異的な免疫反応の誘導や増幅を目指したものであり、本文章では主としてがん抗原特異的ペプチド、ペプチドとキャリアータンパク質との融合タンパク質、及びがん抗原タンパク質等を対象としたがんワクチンの臨床開発における評価事項について言及する。

1.1 背景

活性化リンパ球療法、及び非特異的な免疫活性化療法といった、がんに対する免疫反応の亢進を利用したがん治療の試みは古くから行われていた。しかしながら、これらの非特異的な免疫の活性化による治療の試み法は多くの場合期待された効果は認められず、臨床開発が失敗に終わっている。一方で、ゲノム解析及びがん抗原タンパク質等の網羅的な解析により、がん細胞に特異的に発現するがん抗原の理解が急速に進んでおり、多くのモノクローナル抗体医薬品の開発に貢献している。抗腫瘍抗体医薬品に加え、がん抗原に対する患者の免疫反応を亢進させることによるがん治療の試みが行われている。このがん特異的な獲得免疫の誘導及び増幅を行う治療法として開発されている製品群は多岐にわたっており、がん抗原ペプチド、がん抗原タンパク質、及びがん抗原ペプチド(単鎖ペプチドと長鎖ペプチド)と免疫活性化能の高いタンパク質との融合製品等を始めとして、がん抗原処理した抗原提示細胞としての樹状細胞やがん抗原を導入した患者がん細胞等の細胞治療薬、がん抗原遺伝子を導入したウイルスベクターなどの遺伝子治療薬等が開発されつつある。また、がんに対する免疫の活性化を目的として、免疫賦活化作用のある顆粒球マクロファージコロニー刺激因子（GM-CSF）等のサイトカイン又はアジュバントとがんワクチンの併用投与も試みられている。

がんワクチンの開発にあたり、留意点として例えば以下のような点が挙げられる。

- 目的とするがん抗原が特定されている場合とされていない場合で、免疫応答性の評価方法が変わりうること
- 従来の細胞毒性型の抗悪性腫瘍薬の免疫抑制作用等を有する場合、がんワクチンの期待される作用と相反する臨床効果を持つ場合もあること
- 抗悪性腫瘍薬剤投与後に免疫抑制が惹起される可能性があること

資料２　案２

1.2 がんワクチンの作用メカニズム

多くのがんワクチンで想定されている作用メカニズムは、患者の体内又は体外においてがん抗原等が抗原提示細胞に暴露することにより引き起こされる、生体免疫反応の誘導に依存している。すなわち、抗原提示細胞中でプロセッシングを受けたがん抗原が抗原提示細胞の細胞膜表面で抗原提示され、当該がん抗原に特異的なT細胞応答を誘導する、あるいは既に患者が持っている抗原特異的なT細胞応答性をペプチド等の刺激により増幅させるというものである。このT細胞応答には、がん抗原特異的な細胞傷害活性を持つ細胞傷害性T細胞の誘導及びがん抗原特異的な免疫反応の促進作用をもつヘルパーT細胞の誘導等が含まれる。特にがん細胞に対する傷害作用では、抗原特異的な細胞傷害性T細胞の増幅が薬効の発現に重要とされる。

がんワクチンは、抗原提示細胞を介して誘導されるがん抗原に対する応答性を誘導するものである。これらの抗原提示細胞はヒト白血球抗原（HLA）拘束性にT細胞へ抗原決定基を提示し、提示を受けた細胞傷害性T細胞は同じ抗原決定基を発現している腫瘍細胞を攻撃できるようになると考えられている。ヘルパーT細胞はがん抗原特異的な抗体産生能を持つB細胞応答を補助することもでき、B細胞が産生した抗体による腫瘍細胞死のメカニズムも想定されている。

抗原提示及びそのプロセッシング、リンパ球の活性化、腫瘍細胞死といった宿主免疫系による活性化されがん細胞を攻撃する臨床効果を発揮するまでには、従来の抗がん剤と比べ生体内でかなりの時間を要すると考えられている。したがって、がんワクチンの開発には従来のバイオ医薬品及び化学合成による抗悪性腫瘍薬とは異なり、遅発性の臨床効果を評価できるような試験計画を立案する必要があると考えられる。

1.3 適用範囲

本ガイドラインは治療用がんワクチンを対象とし、がんの予防に用いるワクチンや感染症を対象としたワクチンは対象としない。また、がん細胞を直接攻撃して治療効果を発揮するとされるT細胞やNK細胞を利用した適応免疫製剤についても対象外とする。

なお、上記以外の細胞医薬品や遺伝子治療用医薬品等のがんワクチンについては対象としないが、臨床評価等に当たっては適用できる部分もあると考えられるので、適時参照することが望ましい。

2. ペプチドやタンパク質からなるがんワクチンの品質

資料２　案２

がんワクチンとして用いられる化学合成ペプチドとしては８－９アミノ酸からなる単鎖ペプチドと 25 アミノ酸以上の長鎖ペプチドが用いられている。これらの化学合成ペプチドの品質特性解析では、化学合成薬品としての評価が有用な場合があると考えられる。確認試験では、FT-IR や質量分析による解析が利用可能である。また、純度試験に関してはペプチド合成に用いられる保護基及び保護基の脱離剤、化学合成時における不十分な合成体や分解物等の解析が求められる。規格設定では、日本薬局方に収載されているペプチド医薬品の各条を参考にできるであろう。ただし、分岐鎖を持つ複雑なペプチド製品では、従来の解析手法では十分な構造解析ができない場合があることに留意すべきである。

一方、タンパク質や融合タンパク質では、ICH Q6B に従った組換えバイオテクノロジー応用医薬品としての品質特性解析が求められる。

一方、生物活性の評価としては、in vivo での免疫誘導能を解析することが考えられるが、ヒトでの免疫誘導を動物で評価することは必ずしも容易ではない。また in vitro で免疫誘導能を生物活性として評価する標準的な手法が必ずしも確立されているとはいえない。

In vitro 試験として樹状細胞でのクロスプレゼンテーションが示すことができれば有効性を期待する情報となりえるかもしれないが、手法的な限界も想定されていることも念頭におく必要がある。

3.がんワクチンの免疫学的評価

3.1 免疫応答性評価

がんワクチンの作用メカニズムとしては、投与されたがん抗原刺激により患者のがん細胞に対する免疫反応を誘導ないしは増幅することにより抗腫瘍効果を発現すると考えられている。

また、臨床試験、特に Proof of Concept（POC）等を評価する初期臨床試験では、免疫反応性のモニタリングが重要な評価項目となる。特に薬理学的効果や投与する抗原に対する免疫反応性を示すことが、がんワクチンの有効性を裏づける必要条件となるデータとなっていくことが想定される。また、有効性の評価と相関が認められた場合には、患者選択のための指標となることも想定され、コンパニオン診断薬の開発の必要性も念頭に置く必要があると考えられる。免疫反応性のモニタリングの一つとして、抗原特異的な T 細胞の応答性や標的とするがん抗原に対する液性免疫が測定される。但し、液性免疫に対する応答性のみでは、がん細胞に対する細胞性免疫応答の評価には必ずしも結びつくわけではないため、がんワクチンで想定されている免疫応答を評価する代替指標とすることは困難であろう。

資料２　案２

（1）評価方法について

長鎖ペプチド以上の大きさのがんワクチンの投与に対して期待されている免疫応答には、複数のステップが介在していると考えられている。まず、がん抗原又はがん抗原の一部であるペプチド及びタンパク質等が、樹状細胞などの抗原提示細胞による細胞内プロセッシングを受けた後、HLA クラス 1 依存的に抗原提示される。続いて、抗原提示に対応する特異的な T 細胞受容体をもつ細胞傷害性 T 細胞が活性化され、がん細胞を攻撃する。同時に、HLA クラス 2 依存的にヘルパーT 細胞が活性化され、細胞傷害性 T 細胞の活性化や液性免疫の活性化も行うとされる。がんワクチンの開発に際しては、このような複数の免疫担当細胞の活性化を評価することが重要であり、以下のような複数のアッセイ法を用いて解析することが望ましい。特に、細胞傷害性 T 細胞やヘルパーT 細胞の応答性を区別して評価することが重要と考えられる。

- 目的がん抗原によって増幅する T 細胞サブセット（CD8 陽性や CD4 陽性細胞）の定量
- テトラマーアッセイによる、細胞傷害性 T（細胞 CD8 陽性 T 細胞）及びヘルパーT 細胞（CD4 陽性 T 細胞）細胞数の測定。
- ELISPOT アッセイによる抗原特異的にサイトカインを放出する T 細胞の検出。
- ELISPOT と同様に in vitro で T 細胞を培養し、抗原刺激によるサイトカイン産生と同時に Monensin や Brefeldin-A 等の細胞内タンパク質輸送を阻害する薬剤を用いてサイトカインの細胞外への放出を阻害することにより細胞内に蓄積され、細胞内に蓄積したサイトカインを蛍光標識抗体で標識し、フローサイトメトリーを用いて解析することにより抗原刺激に応答する細胞を検出する（フローサイトメトリーアッセイ）。CD8 や CD4 の発現を同時に解析することも可能である。
- がんワクチンとして長鎖ペプチドやタンパク質を用いる場合、免疫応答によりどのようなペプチドが認識されるようになるかあらかじめ特定できない場合がある。このような場合に免疫応答により認識されるペプチドを特定するためにいくつかの方法が用いられている。例えばがん抗原の一次配列を網羅するようなペプチドライブラリーを用いて T 細胞を刺激し ELISPOT アッセイを行うなどが行われている。ペプチドライブラリーの感度等について十分な評価を行う必要がある。
- ペプチド特異的 CD8 陽性細胞や CD4 陽性細胞の末梢血中の頻度が極めて低い可能性がある。高感度に測定するために濃縮操作なども行われている。濃

縮操作によってもとの血液中の比率と異ならないことを確認する必要がある。

細胞応答性を解析する以上のアッセイ法は単なる例示であり、がんワクチンによって引き起こされる免疫応答の解析で実施しなければならないアッセイというわけではない。特にがんワクチンの免疫応答性の評価系については開発が急速に進歩しており、より最適な手法を用いることも可能となることが想定され、また対象としている抗原によっても利用可能な試験法が異なると考えられる。

また、免疫応答に寄与するT細胞数は末梢血中では極めて少ないとされ、上記のモニタリング法の感度を上げる様々な工夫が試みられている。高感度化した試験を実施する場合には試験法が適切にT細胞集団を反映する方法であることを示す必要がある。

(2) 開発戦略について←より良い案があればお願いします。

特定のTCRをもつT細胞に反応するHLAクラス1及び2に抗原提示されるペプチドを結合させたテトラマープルーブを用いる解析により、がん抗原に反応するHLA型の特定やHLA拘束性のがんワクチンの活性評価が可能になるかもしれない。がんワクチンの免疫応答性の評価ではどのようなHLA型の患者に有用な治療であるのかを明らかにすることが重要と考えられる。

(3) 評価方法のバリデーションについて

抗腫瘍効果に関連すると考えられる免疫応答性を評価するためのアッセイ法の開発や標準化が必要とされる。特に、生細胞の反応性を解析することから、検体の採取から輸送による影響や凍結して保存する場合の凍結操作の影響について評価をしておく必要がある。また、がんワクチンの投与を受けた患者の経時的な変化を測定するために、異なる日に測定されたT細胞の応答性を比較可能なように測定法の標準化ができていることを示しておく必要がある。

開発したアッセイ法のバリデーションでは、試験実施施設が異なっても、免疫応答のバラツキを十分コントロールできることを示す必要がある。アッセイ条件、感度と特異性のコントロール、in vitroでのT細胞の増幅工程、陽性及び陰性コントロールの設定、カットオフ値の設定、標的分子による対象患者の選択の要否、試験結果を解析するための統計分析手法等については、臨床試験を開始する前に明確にしておく必要がある。