Local Modulation of Immune Receptor Function to Enhance Immune Responses to Dendritic Cell Vaccination in Subjects with Triple Negative Breast Cancer

Protocol 1208-1180

NIH Recombinant DNA Advisory Committee Meeting

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Clinical Research Team

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- **Kimberly Blackwell, M.D. (Collaborator)**
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  - Breast Cancer Medical Oncologist
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- **Shelley Hwang, M.D. (Collaborator)**
  - Professor of Surgery, Duke University
  - Chief, Breast Surgical Oncology, Duke Cancer Institute

Dendritic Cell-Based Cancer Immunotherapy

- Numerous early phase clinical trials
  - Variety of cancers, including breast
  - Many in patients with advanced metastatic disease
- Well tolerated with minimal toxicity
- Immune and clinical responses variable
  - Most trials have focused on antigen delivery
  - Few trials have focused on overcoming immune regulatory pathways

Systemically Administered Immune Modulators Can Augment Immune Responses, **BUT**:

- Anti-CTLA-4 mAbs
  - Stimulate autoimmune responses in mice
  - Stimulate autoimmunity in humans
    - Severe, sometimes fatal, autoimmune colitis
    - Dermatitis
    - Other autoimmune effects
    - Approved by FDA for metastatic melanoma
    - Being evaluated in numerous clinical trials as adjuvant therapy
- Anti-GITR mAbs
  - Stimulate autoimmune responses in mice

Our Proposed Approach

- Vaccinate triple negative breast cancer (TNBC) patients with autologous monocyte-derived dendritic cells (DCs)
- Use RNA transfection to load the vaccine DCs with TNBC antigens
- Co-transfect these DCs with RNA encoding soluble immune modulators to
  - Locally deliver immune modulators targeting CTLA-4 and GITR to lymph nodes at the site of the interaction of tumor antigen-loaded DCs and T cells
  - Augment anti-tumor immune responses using local immune modulator delivery while avoiding systemic autoimmunity

Proposed Immunotherapeutic Trial in Triple Negative Breast Cancer

- Subjects with T2-4/N0-3/M0 triple negative breast cancer
  - Vaccination after surgery and adjuvant radiation/chemotherapy
- Autologous monocyte-derived dendritic cell vaccine
  - Transfected with RNAs encoding TNBC antigens
    - EGFR, MUC1, Mage-3
  - Co-transfected with RNAs encoding immune modulators
    - No RNA (Study Arm A)
    - sGITRL fusion protein RNA (Study Arm B)
    - Anti-CTLA4 mAb RNA (Study Arm C)
    - sGITRL fusion protein + Anti-CTLA4 mAb RNAs (Study Arm D)
- Susan G. Komen for the Cure support for this proposed Phase I trial

Rationale for Targeting TAAs EGFR, MUC1 and MAGE-3 in TNBC

- EGFR is over-expressed by over 60% of TNBCs.
- MUC1 is over-expressed by more than 70% of breast cancers.
  - Over-expressed in 92% of TNBCs
- MAGE-3 is overexpressed in 26% of TNBCs.
  - MAGE gene activation seen in metastatic lesions even when the primary breast cancer is negative for MAGE expression.
- These selected TAAs are also immunogenic
  - Low levels of immune responses against all three of these proteins have been detected in patients with cancer.
  - We anticipate that vaccination of TNBC patients with DCs transfected with RNAs expressing these three TAAs will boost these immune responses.
- By modulating immune receptors, as described in our proposal, we anticipate that immune responses against these three TAAs will be further enhanced in vaccinated TNBC patients.

Specific RAC Reviewer Comments

Dr. Hammarskjold-

What is the expression of the TAAs in normal tissues?

Dr. Kiem-

What other tissues are expected to express the targeted TAAs and what responses to other tissues have been observed in previous studies targeting these TAAs?

Normal Tissue Expression of TNBC TAAs Targeted in this Proposal

- EGFR
  - Expressed in normal tissues at low levels (pancreas, liver, lung, and skin)
  - mAbs targeting EGFR are used clinically to treat cancer
    - Side effect of skin rash (the development of which directly correlated with survival benefit).
- MUC1
  - Expressed on the cell surface at low levels in most epithelial cells of the breast, respiratory, and genitourinary tracts.
  - Several MUC1-loaded DC-based cancer immunotherapy trials have been performed, without any serious adverse reactions.
  - Open vaccine trial listed on *clinicaltrials.gov* in which patients with stage I and II (ie T1-2/N0-1) TNBC are being vaccinated with adjuvant combined with a MUC-1 peptide.
- MAGE-3
  - Cancer-testes antigen
  - Expression in normal tissues limited to testes

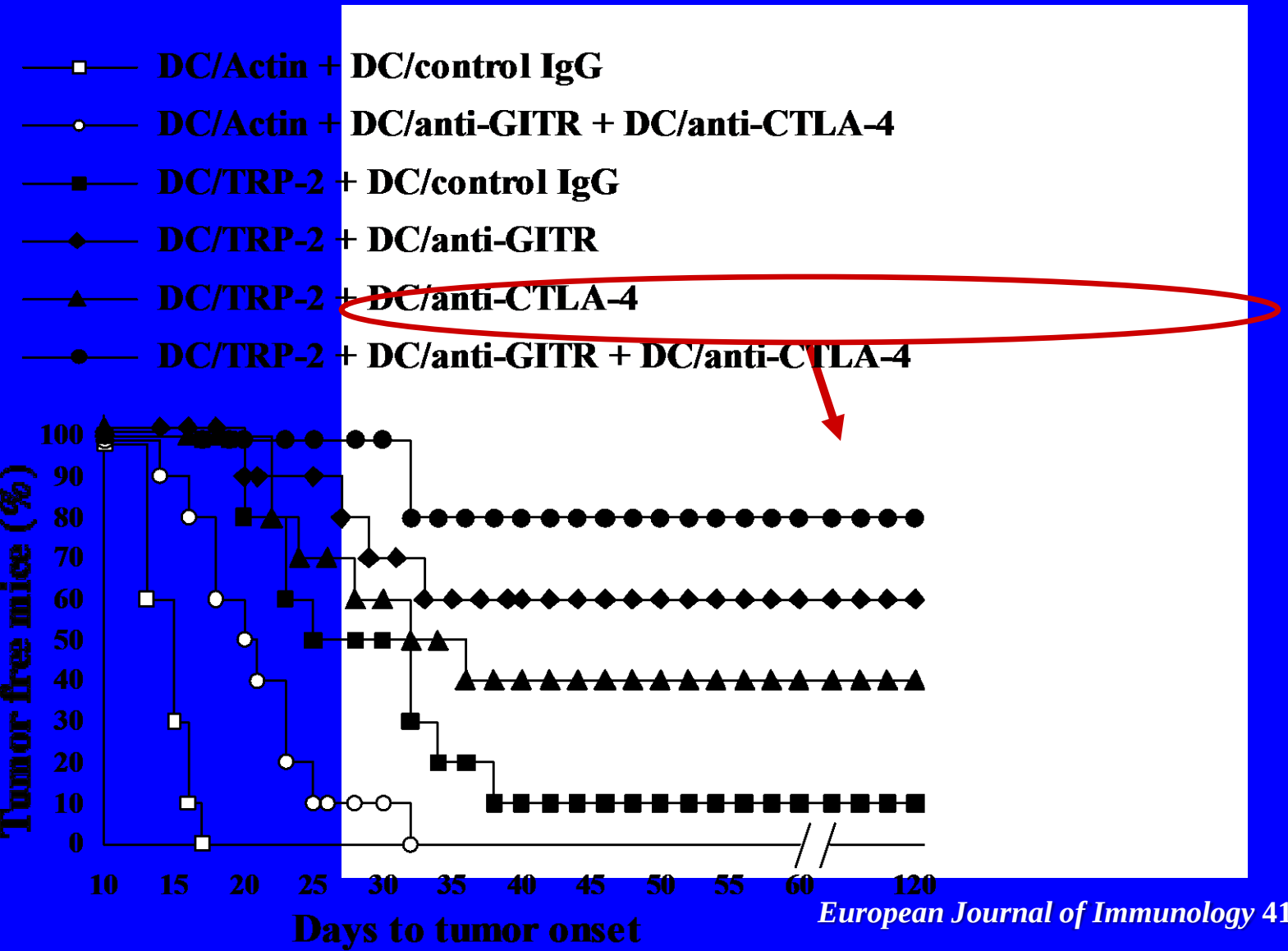
RNA Encoding Immune Modulators

- Anti-mouse CTLA-4 and anti-mouse GITR mAbs used for murine studies
  - Genes encoding the heavy and light chains cloned from commercially available hybridomas
  - RNAs generated using *in vitro* transcription
- For human studies
  - Anti-CTLA-4 mAb
    - Heavy and light chain genes cloned from murine hybridoma
    - Murine constant regions replaced with human sequences = "humanized"
  - Soluble GITR-ligand (sGITR-L)
    - No anti-human GITR mAb available
    - Human GITR-ligand cloned and used to construct a soluble GITR-L fusion gene
  - Clinical use of these RNAs in melanoma Phase I trial
    - Previously reviewed by the RAC without the need for public comment (Protocol # 0906-983)
    - IND application approved by the FDA (IND # BB-14100)

Preclinical Studies That Provide the Rationale for our Clinical Trial Proposal

- Co-transfection of TAA RNA-transfected DCs with RNAs encoding anti-GITR and anti-CTLA-4 mAbs enhances the induction of therapeutic anti-tumor immunity and improves survival in a murine melanoma model.
- The immune-modulatory effects of DCs transfected with RNAs encoding anti-GITR occur locally at the site where TAA RNA-transfected DCs stimulate TAA-specific immune responses and are not due to systemic secretion of the immune modulator. Autoimmune effects avoided.
- Human monocyte-derived DCs transfected with RNAs encoding either a humanized anti-CTLA-4 mAb or a soluble human GITR-L fusion protein secrete functional immune-modulator proteins.
- Breast cancer antigen-specific immune responses induced by human monocyte-derived DCs transfected with RNAs encoding TNBC TAAs are enhanced by DC co-transfection with RNAs encoding soluble immune modulators GITR-L and humanized anti-CTLA-4 mAbs, and the immune-enhancing effects of using both immune modulators are additive.

Tumor-specific immunization synergizes with local immune receptor modulation to enhance tumor immunity



European Journal of Immunology 41:3553-3563 (2011)

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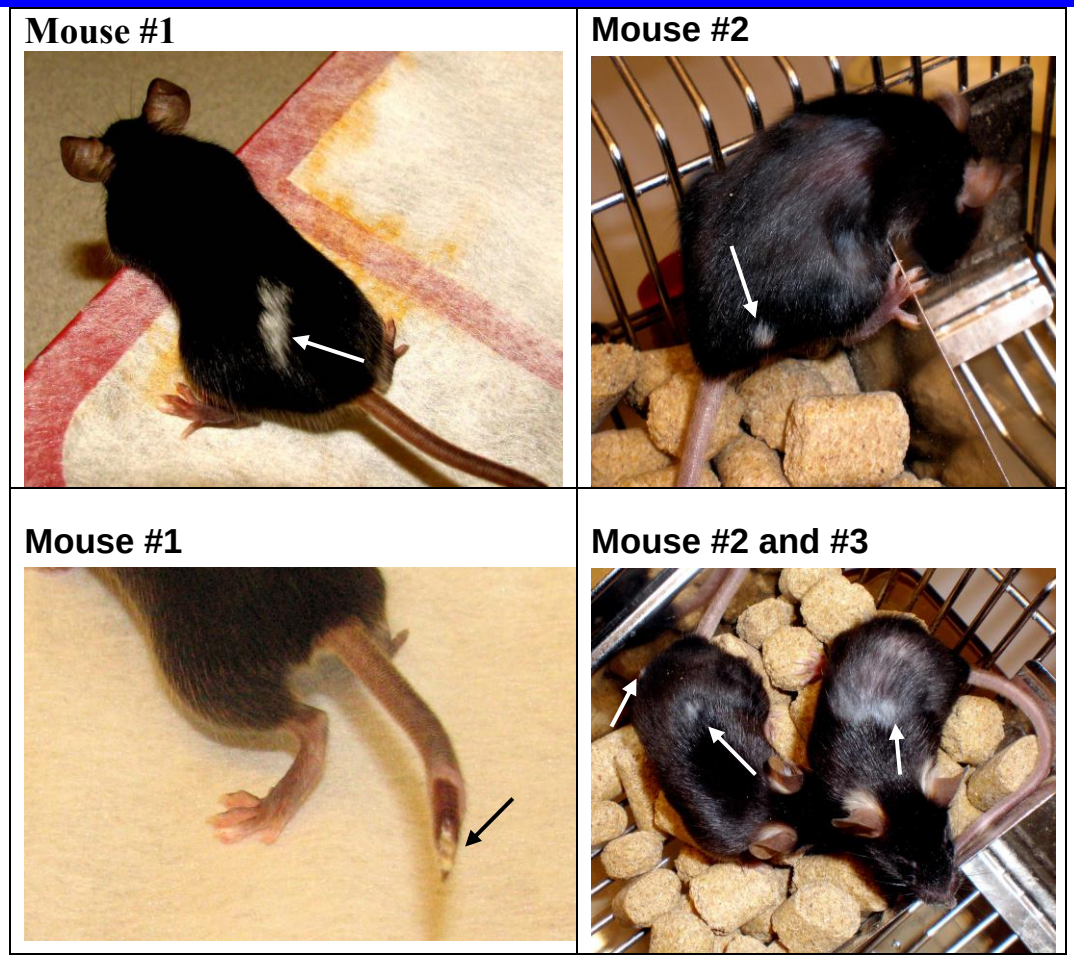
Autoimmunity in Mice Receiving Systemic Anti-GITR mAb Treatment

(3 of 14 mice vs 0 of 55 mice receiving DC transfected with anti-GITR RNA, $p<0.05$)



Cancer Gene Therapy **16:900 (2009)**

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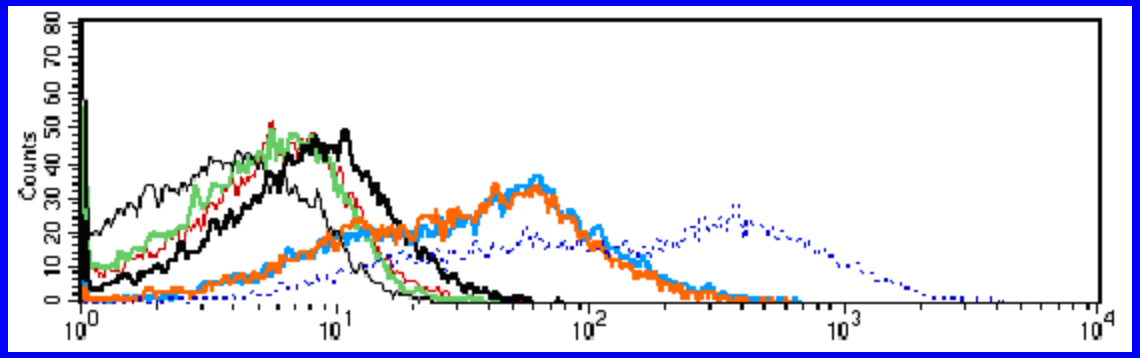
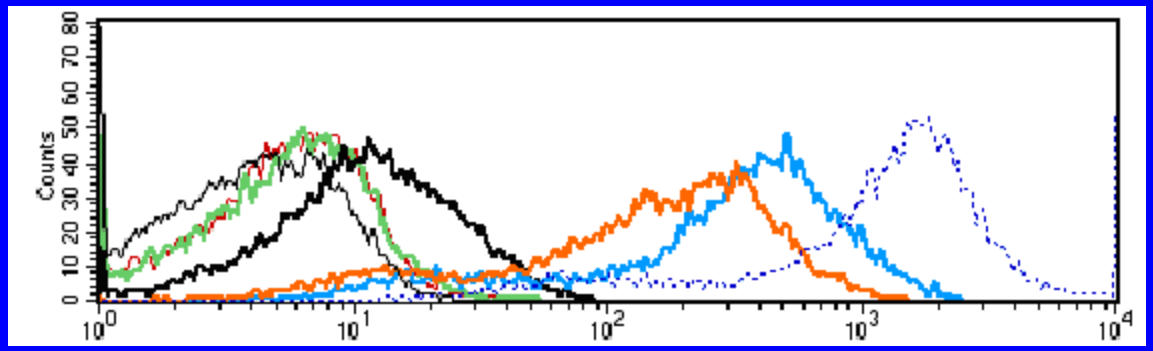
DC Secrete Soluble Immune Modulators for Up to 48 Hours Following RNA Transfection

A Supernatant from anti-CTLA-4 RNA-transfected DC



Relative Fluorescence of Binding to CTLA-4-expressing cells

B Supernatant from soluble GITR-L RNA-transfected DC



Relative Fluorescence of Binding to GITR-expressing cells

Supernatant Time Point

- unstained
- day 6 supernatant
- 72 hr supernatant
- 48 hr supernatant
- 24 hr supernatant
- 6 hr supernatant
- commercial antibody

European Journal of Immunology 41:3553, 2011

Preclinical Studies That Provide the Rationale for our Clinical Trial Proposal

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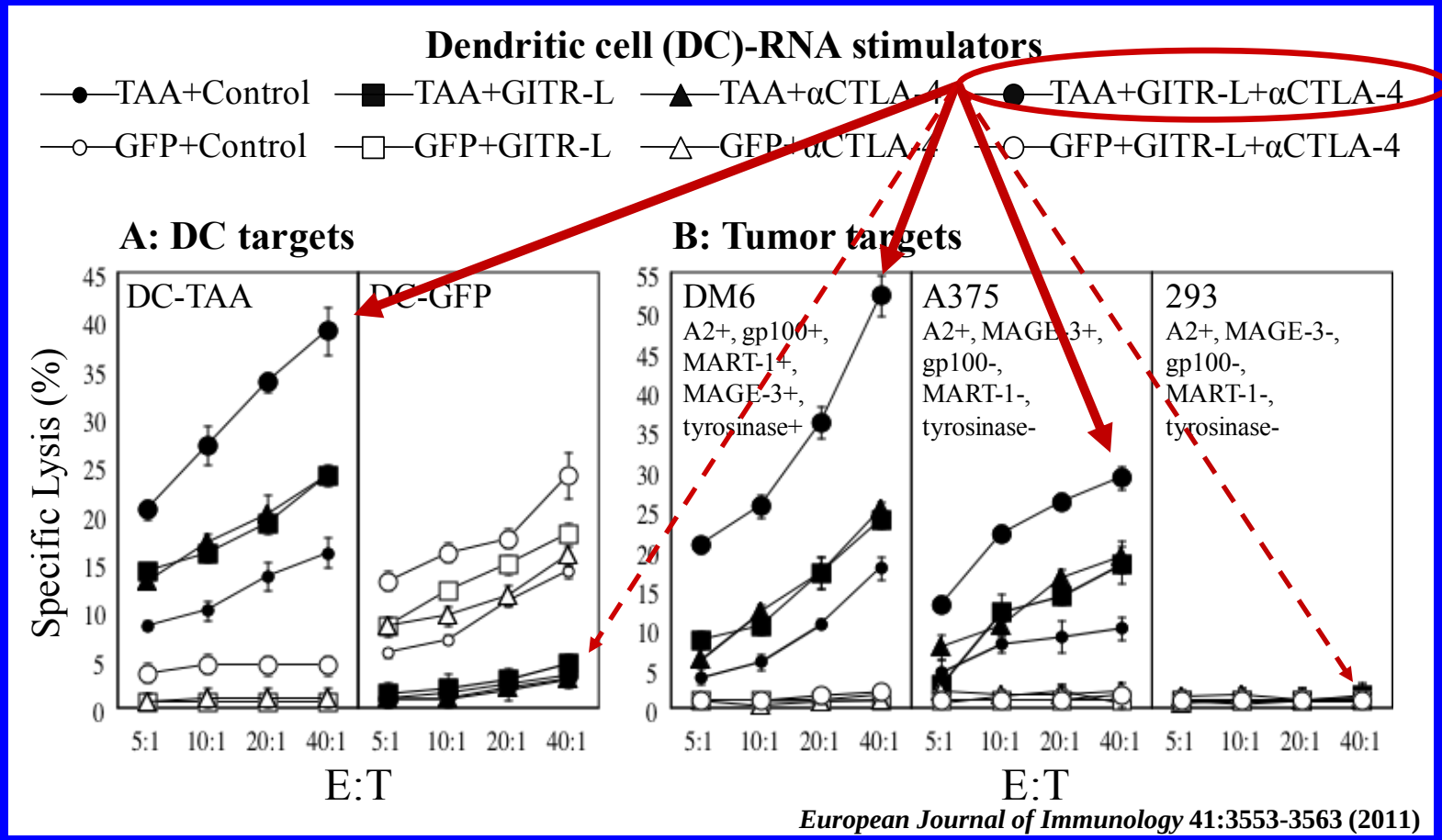
sGITR-L and anti-CTLA-4 mAb RNA Transfection Enhances the Immunogenicity of Melanoma TAA RNA-Transfected DC

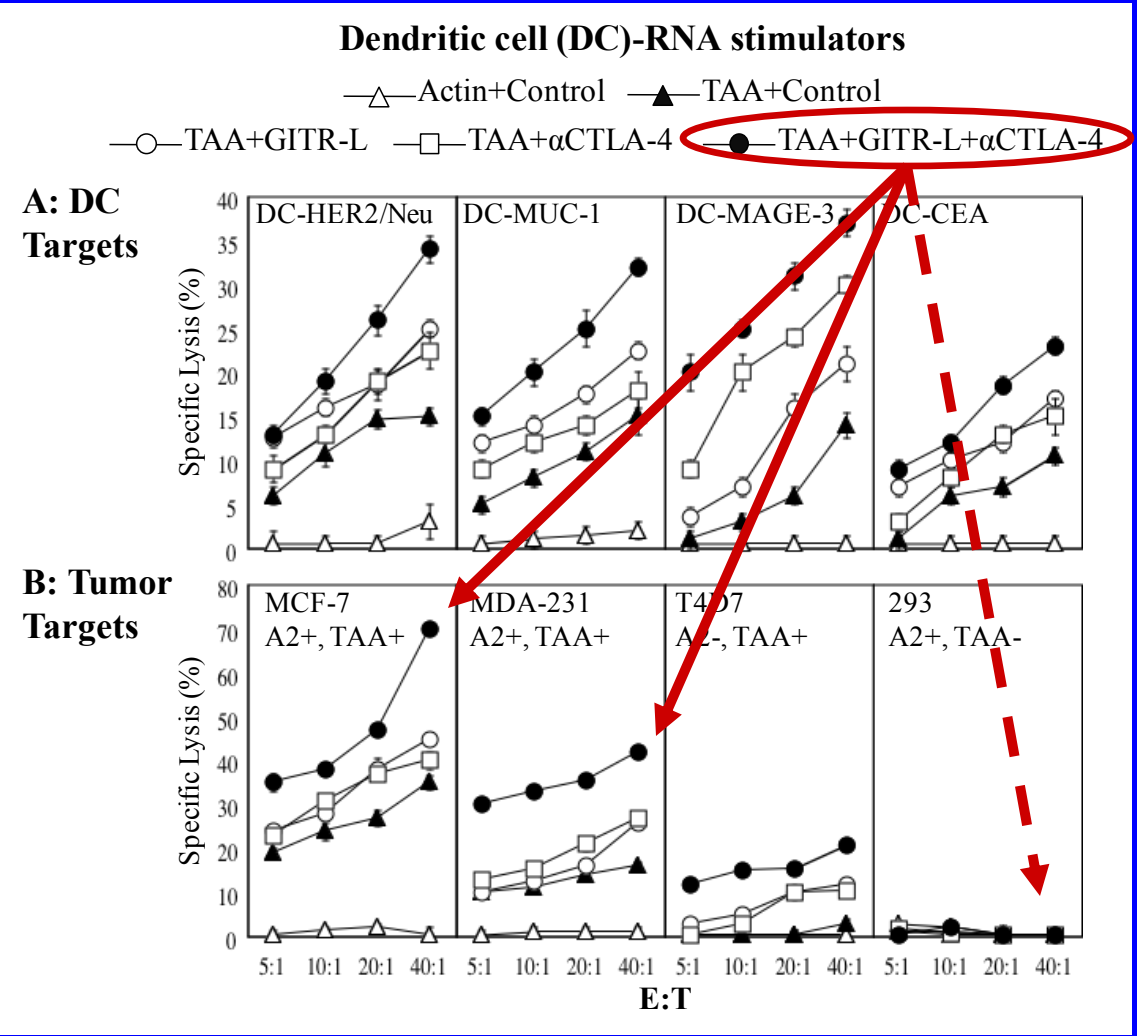


European Journal of Immunology **41:3553-3563 (2011)**

No background lysis against control RNA transfected DCs

No background "autoimmunity" against control TAA-negative 293 cells

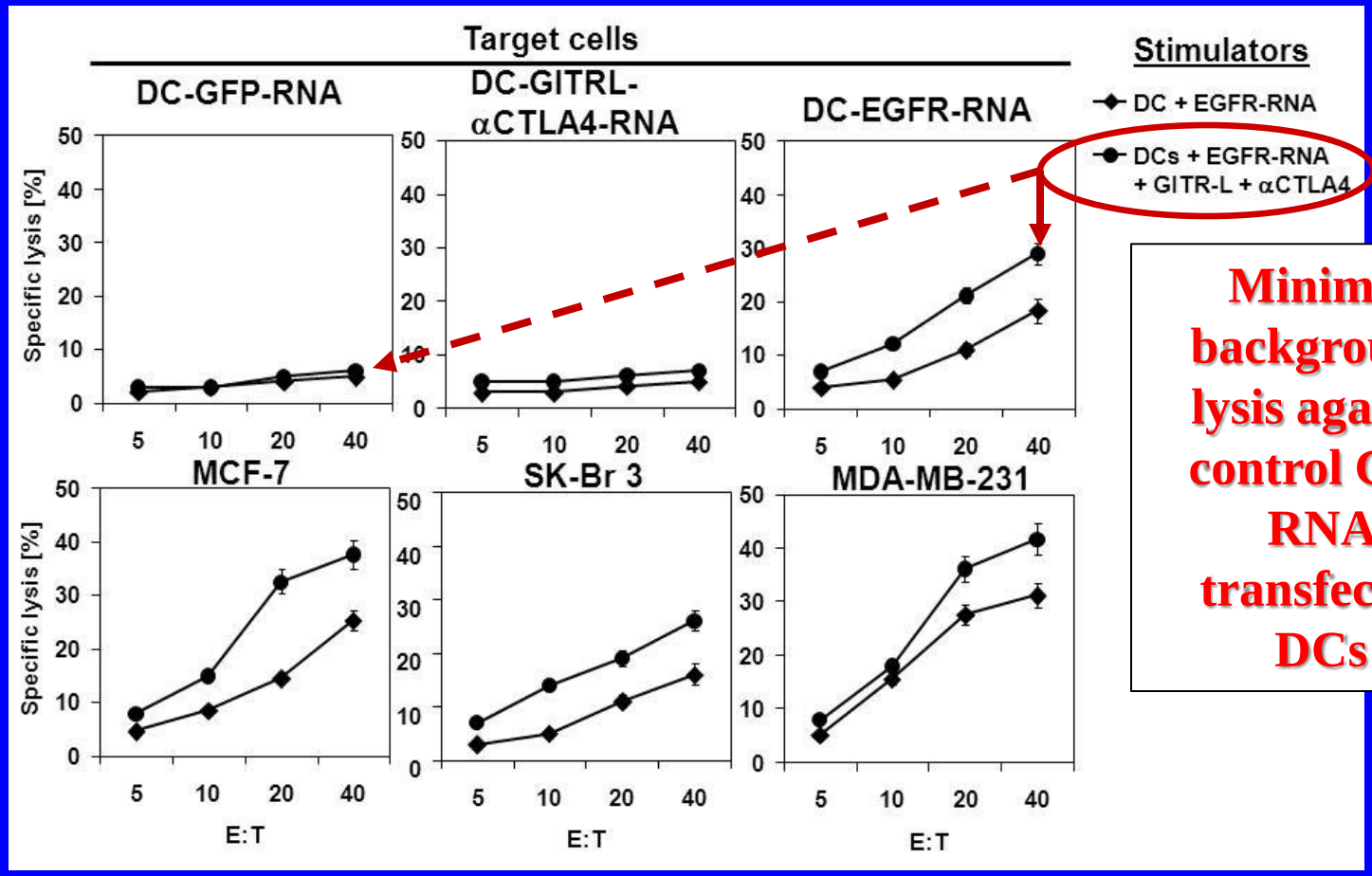
sGITR-L and anti-CTLA-4 mAb RNA Transfection Enhances the Immunogenicity of Breast TAA RNA-Transfected DCs



No background "autoimmunity" against control TAA-negative 293 cells

European Journal of Immunology **41:3553, 2011**

CTL activity of T cells stimulated by EGFR RNA-transfected DC is enhanced by co-transfection with RNAs encoding GITR-L and anti-CTLA-4 mAb



Minimal background lysis against control GFP RNA transfected DCs

Specific RAC Reviewer Comments

Dr. Dresser-

"Would it be reasonable to exclude women with node-negative disease, since this group has significantly better survival rates than do women with node-positive disease?"

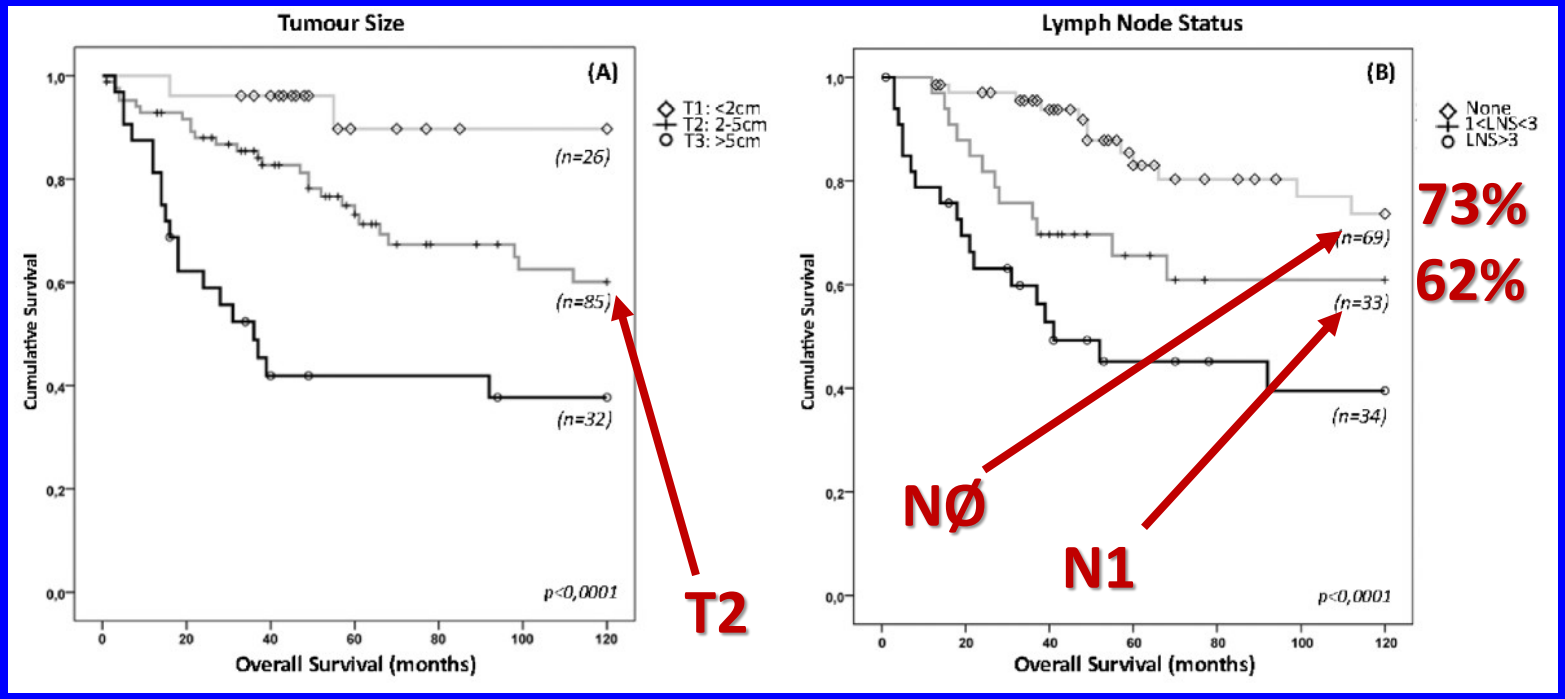
Dr. Kiem-

Main comment is regarding the inclusion of N0 patients. Given that this is a first in human study it would be helpful to better justify the enrollment of N0 patients – could this study be initiated with more advanced patients, what is the downside of that? It would also be helpful to provide survival info for TNBC with current therapy for the different tumor stages in both protocol and consent form.

Patient Selection

- Patients with T2-4/N0-3/M0 TNBC
- Vaccination following completion of standard of care surgery, radiation, and chemotherapy
- T2-4 subjects with node-negative TNBC not excluded:
  - Risk of vaccine toxicity is expected to be low
  - These patients receive chemotherapy, regardless of nodal status
    - The patients are at high risk for metastatic disease
    - This population might benefit the most from effective adjuvant immunotherapy
  - A large number of node-negative subjects have distant disease at the time of surgery
    - 5 year survival for patients with node negative TNBC is ~ 73%
    - 5 year survival for TNBC patients with involvement of 1-3 nodes is ~ 62%
    - Reported propensity of TNBC to metastasize not only by lymphatic but also through hematogenous spread.

Survival in Patients with TNBC Based on Tumor Size and Nodal Involvement



Albergaria *et al. BMC Cancer* 2011 **11**:299

In Triple Negative (Basal-like) Breast Cancers, Smaller Tumors Do Not Translate into Improved Survival

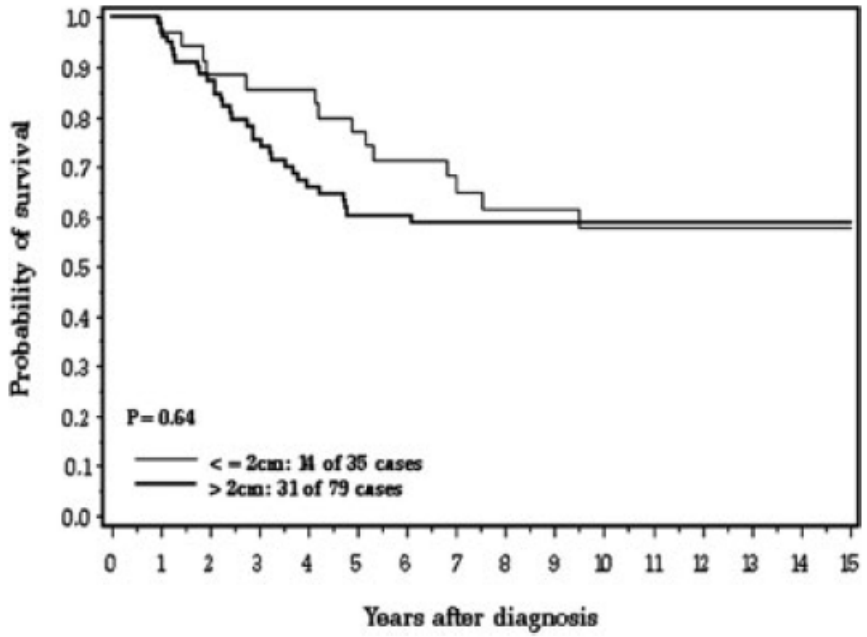


FIGURE 1. Rates of breast cancer-specific survival are illustrated for women with basal-like breast cancers stratified by tumor size.

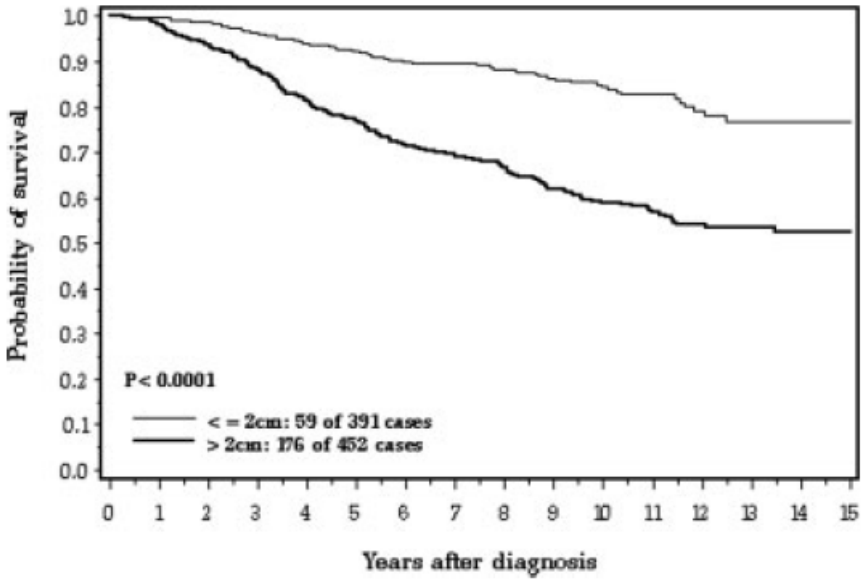


FIGURE 2. Rates of breast cancer-specific survival are illustrated for women with nonbasal breast cancers stratified by tumor size.

Cancer 2009;115:4917–23.

Patients with Node-Negative Triple Negative (Basal-like) Breast Cancers Have a Worse Prognosis Than Those with Negative Nodes and Other Types of Breast Cancer

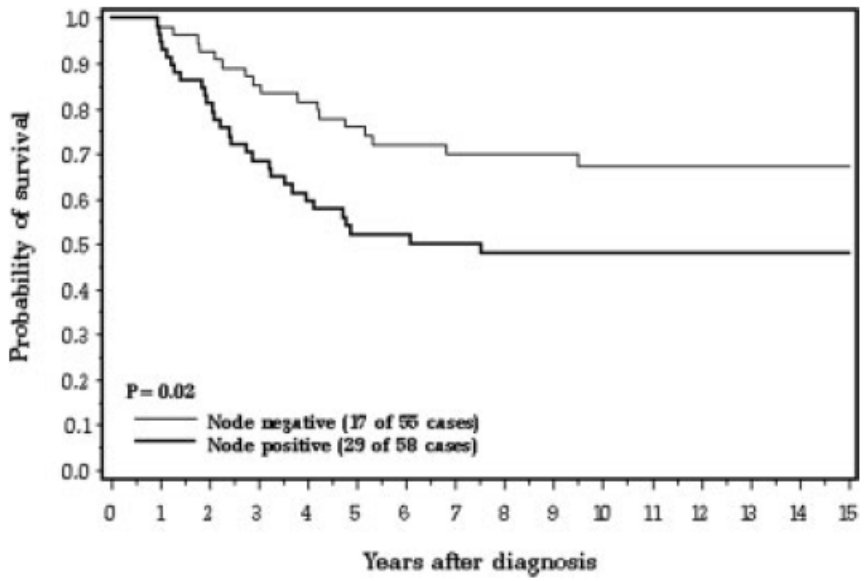


FIGURE 3. Rates of breast cancer-specific survival are illustrated for women with basal-like breast cancers stratified by lymph node status.

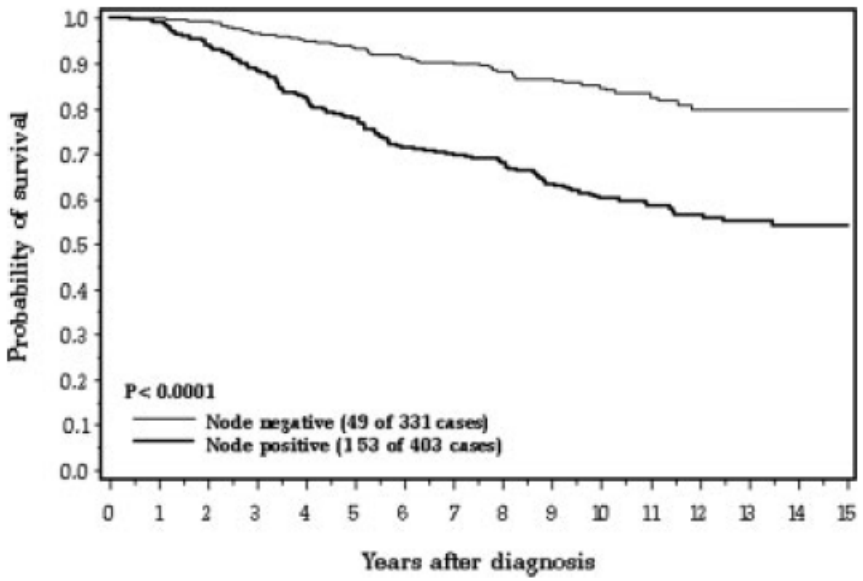


FIGURE 4. Rates of breast cancer-specific survival are illustrated for women with nonbasal breast cancers stratified by lymph node status.

Cancer 2009;115:4917–23.

Specific RAC Reviewer Comments

Dr. Hammarskjold-

Consent Form

Page 10: “ What alternatives are there to participation in this study?” This paragraph as written, makes it sound like TNBC is more or less a “death sentence”. As stated above, the prognosis is more hopeful for some patients. Can this paragraph be changed to reflect this?

Alternatives to Study Participation

- Subjects will only be vaccinated after completing standard of care surgery and adjuvant radiation and chemotherapy
  - Additional adjuvant therapy is available for patients with ER/PR+ breast cancers (tamoxifen, aromatase inhibitors)
  - Additional targeted therapy is available for patients with Her2/neu+ breast cancers (herceptin)
  - No other adjuvant therapy or targeted therapy is currently available to improve the prognosis of and reduce recurrence in patients with TNBC
- Immunotherapy offers a targeted approach to potentially improve the outcome for TNBC patients
- Short of participation in this proposed trial, or in another clinical trial, these patients are followed clinically with no further therapy.

Specific RAC Reviewer Comments

Dr. Dresser-

Consent form, page 2, "Why is this study being done?" Add: This is the first time that this vaccine will be tested in humans.

Consent form, page 10, "Are there benefits to taking part in this study?" Add: Treating your disease is not a purpose of this study. It is very unlikely that getting the study vaccine will improve your health.

Dr. Hammarskjold-

Page 10: "If you agree to take part in this study, there may not be direct medical benefits to you". This is a "first in man" safety study. Thus this sentence should reflect this and the paragraph should be changed. I would propose " a direct medical benefit to you is unlikely. However, we hope the information learned…..

Dr. Kiem-

It would also be helpful to provide survival info for TNBC with current therapy for the different tumor stages in … consent form.

Informed Consent Form Concerns

DUKE UNIVERSITY HEALTH SYSTEM

Consent To Participate In A Research Study
LOCAL MODULATION OF IMMUNE RECEPTOR FUNCTION TO ENHANCE IMMUNE RESPONSES TO DENDRITIC CELL VACCINATION IN SUBJECTS WITH ~~METASTATIC~~ TRIPLE NEGATIVE BREAST CANCER

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to test the safety of a new investigational breast cancer vaccine strategy. **This is the first time that this vaccine will be tested in humans.** This vaccine is designed to boost the immune system against triple negative breast cancer cells within your body. It is believed that the body's immune system can attack tumor cells and kill them. In most patients with advanced cancer, including triple negative breast cancer, however, the immune system is not able to adequately destroy the cancer. Our laboratory research indicates that immune responses to vaccines can be improved by adding immune stimulators that bind to and block specific inhibitory receptors. These immune stimulators can be delivered locally to the site of immune stimulation by injecting immune cells co-loaded with RNA (nucleic acids) designed to make tumor proteins and with RNAs designed to direct the secretion of these immune stimulators.

This study is "investigational" because the study vaccine is being tested in research studies and is not approved as therapy by the U.S. Food and Drug Administration (FDA).

← In response to Dr. Dresser

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

Treating your disease is not the purpose of this study. If you agree to take part in this study, there may not be direct medical benefit to you. We hope the information learned from this study will benefit other patients with triple negative breast cancer or other cancers in the future.

← In response to Dr. Dresser

"a direct medical benefit to you is very unlikely"

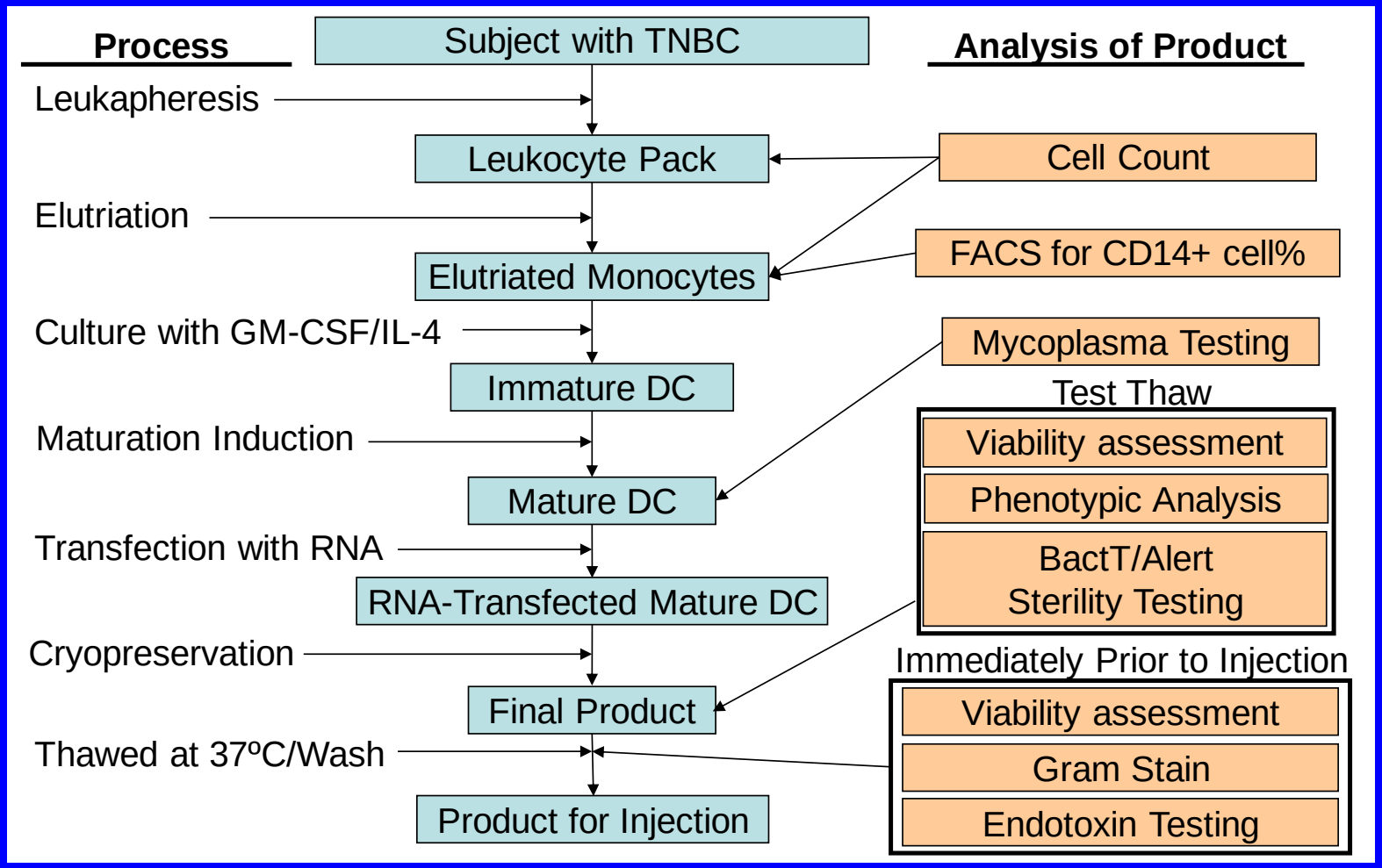
WHAT ALTERNATIVES ARE THERE TO PARTICIPATION IN THIS STUDY?

For triple negative breast cancer patients who have completed surgical therapy, adjuvant radiation therapy, and adjuvant chemotherapy, there are no ~~other~~ **additional** known therapies that have been developed that have been found to **further** prolong your life. Instead of being in this study, you could also participate in another research study. Short of study participation, patients with triple negative breast cancer who have completed standard therapy, ~~usually consisting of surgery followed by adjuvant radiation and chemotherapy, simply must "watch and wait."~~**are followed clinically and receive additional therapy only if their cancer recurs.**

Please talk to your doctor about these and other options.

← In response to Dr. Hammarskjold

Vaccine Manufacturing Process



Specific RAC Reviewer Comments

Dr. Kiem-

Please expand on section M-II-B-2-b-(6). Is the gene expressed in cells other than the target cells?

Response-

- Only autologous monocyte-derived DCs will be transfected with RNAs *ex vivo*.
- No other host cells can therefore be transfected with these RNAs.
- RNA does not have any transforming potential

DC Quality Control

- DC Culture Medium - Mycoplasma testing
- Test thaw of one vial of DCs
  - Bacterial and Fungal Testing
    - BacT/Alert System
  - DC phenotype using FACS
  - **DC TAA and immune modulator expression using intracellular FACS**
- Testing of each vaccine dose on day of vaccination
  - Endotoxin level
  - Gram stain
  - DC viability

Specific RAC Reviewer Comments

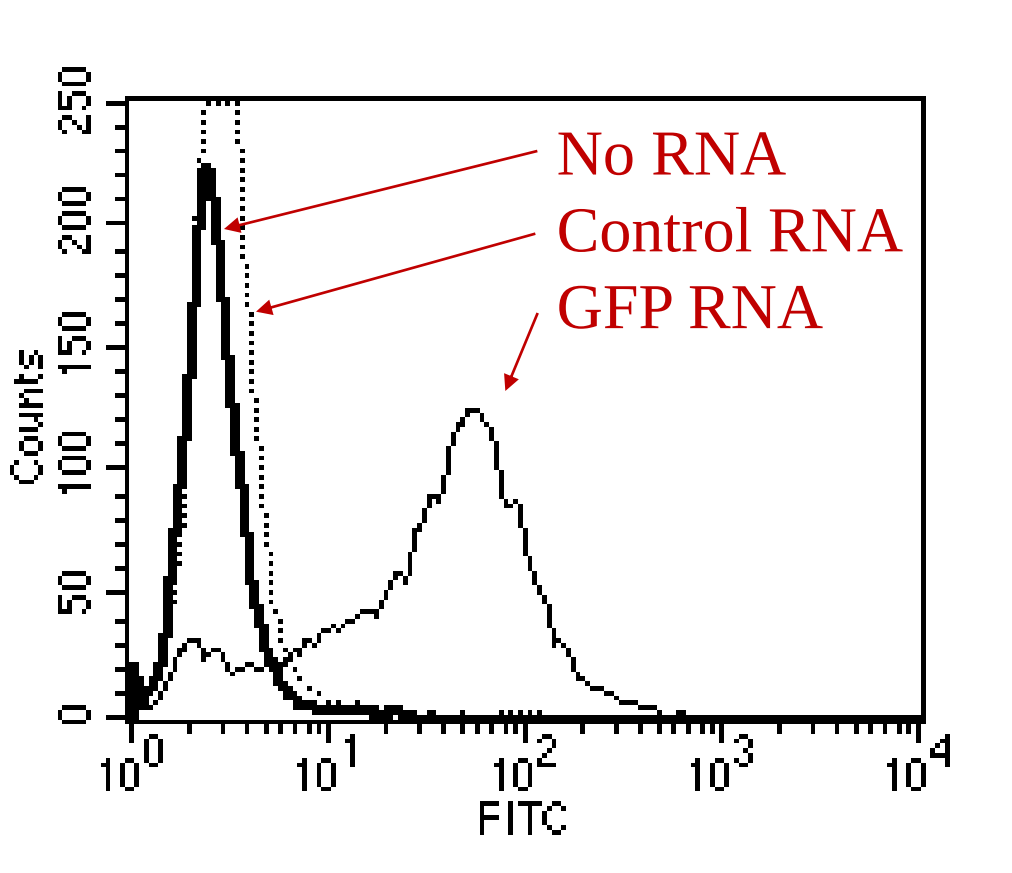
Dr. Hammarskjold-

What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined? Will expression levels be determined for the different TAAs etc. before "vaccination"? If so, by what method? Also, is data now available from the previously approved DC-based trial (RAC Protocol #0708-874)?

Dr. Kiem-

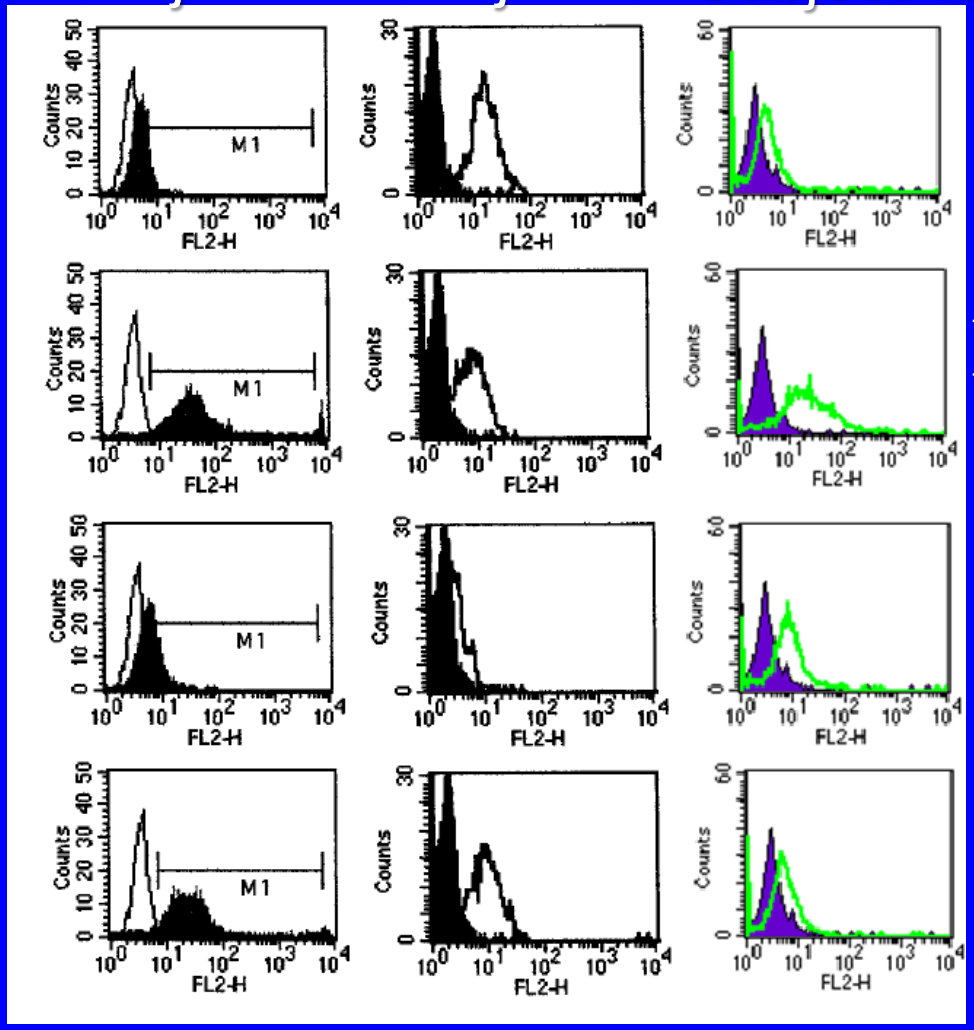
… How will they assess the electroporation efficiency and thus DC modification – how consistent are the electroporation efficiencies within one arm and how will this be assessed? Different levels of expression could affect the immune responses elicited in the different arms of the study.

As Determined Using GFP RNA, the Efficiency of RNA Electroporation of DCs is Routinely Approximately 70%



Assessment of DC Melanoma Vaccine TAA Expression after RNA Transfection (RAC Protocol #0708-874)

Subject 1 Subject 5 Subject 12



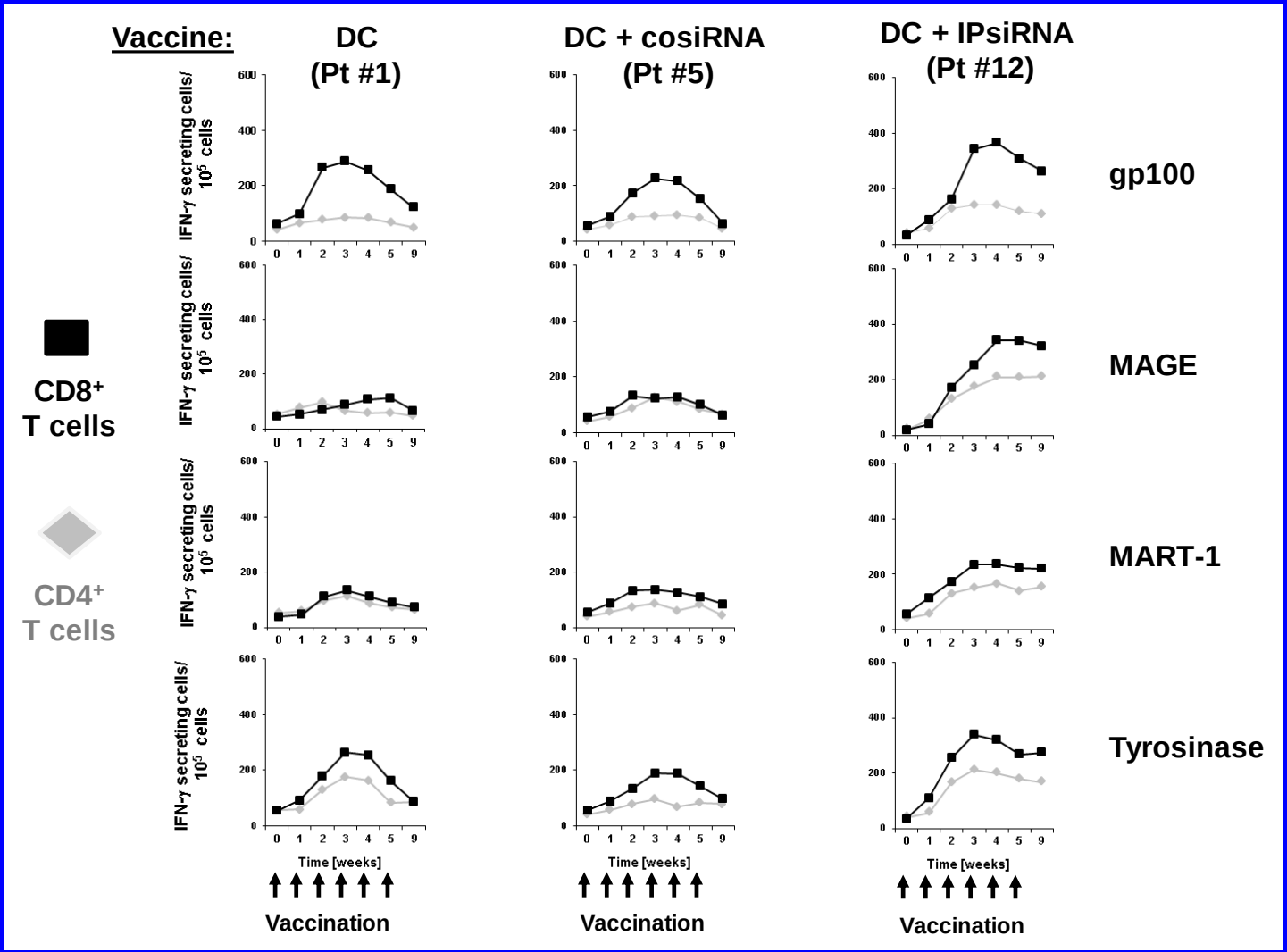
gp100

Mage-3

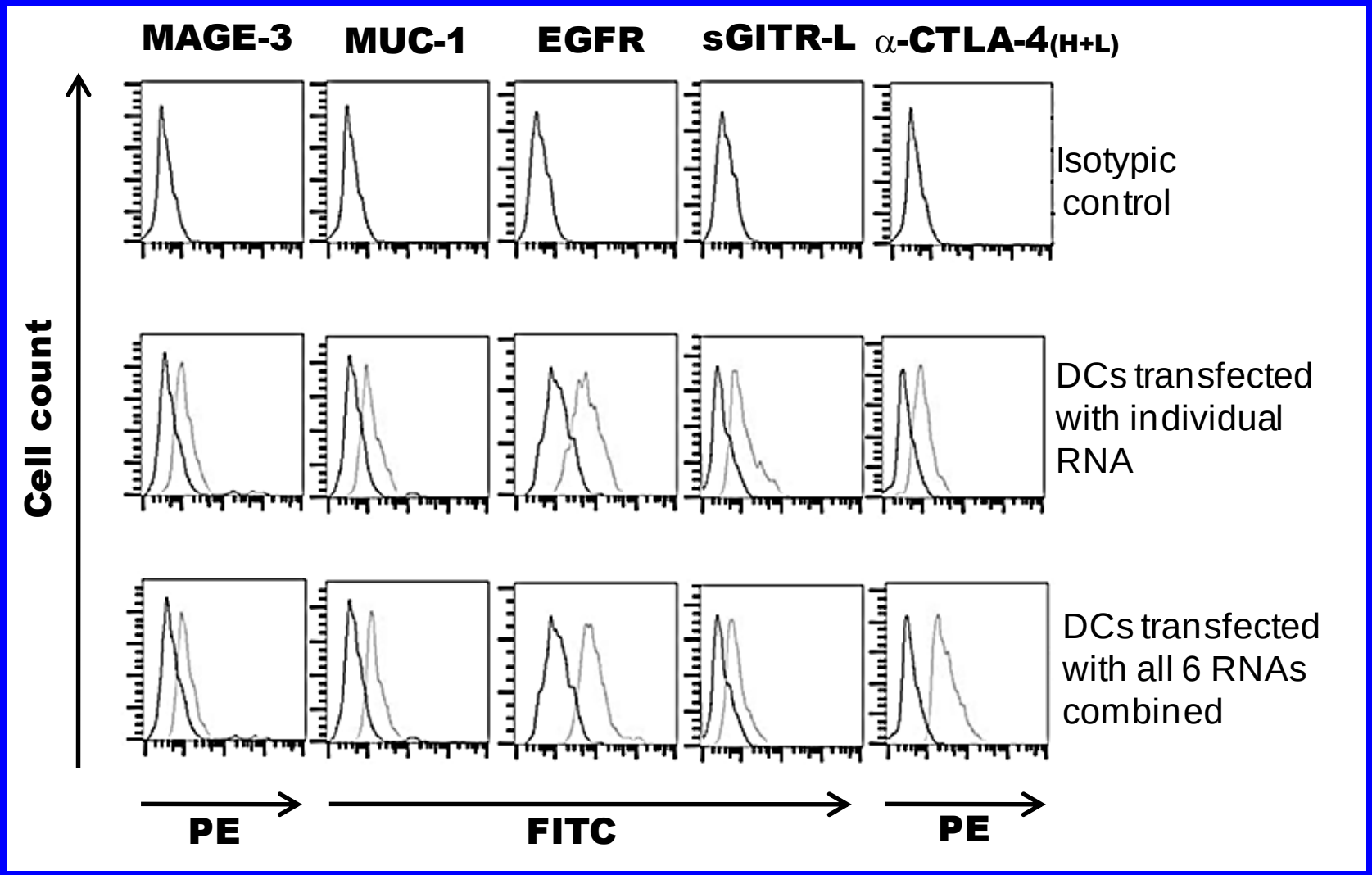
Mart-1

tyrosinase

Melanoma TAA-Specific Immune Responses Following Vaccination Did Not Correlate with DC TAA Expression Levels



Transfection with Multiple RNAs Does Not Diminish the Translation of each RNA



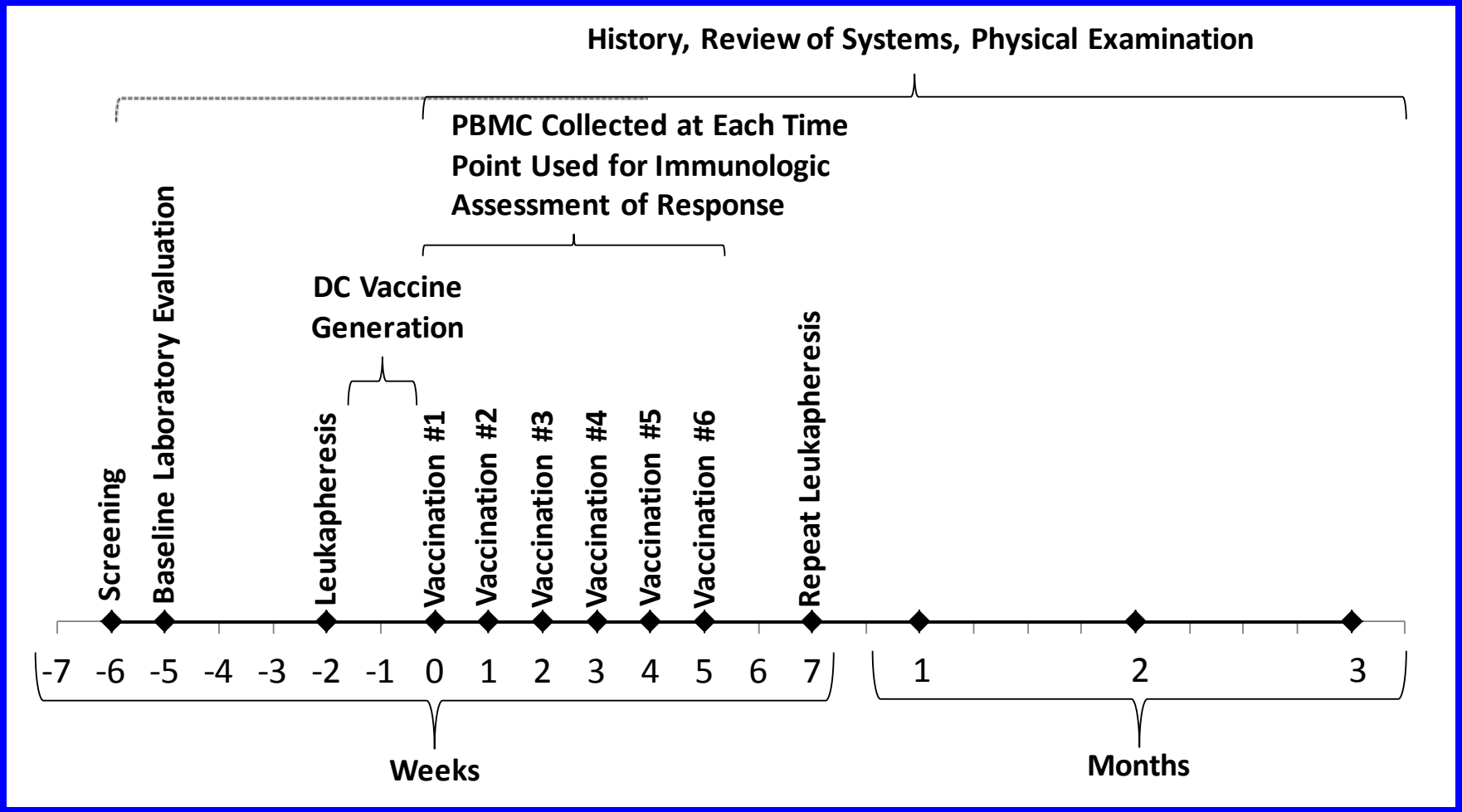
TAA and Immune Modulator Expression Analysis - Summary

- TNBC TAA expression will be assessed using TAA-specific mAbs and intracellular flow cytometry
- Immune modulator expression will be assessed similarly
- As approved by the FDA for a similar trial in melanoma:
  - No “cut off” expression value will be set
  - Expression levels measured and will be compared to immune responses

Injection of Dendritic Cell Vaccine

- Vaccination
  - Six vaccinations at weekly intervals
  - Administered by intranodal injection under ultrasound guidance
    - Well tolerated in first two subject in another approved Phase I trial in melanoma
  - Rotation of between contralateral axillary and inguinal nodal basins

Clinical Vaccination Time Line for Each Subject



Assessment of Response to Vaccination

- The **primary endpoint** of this proposed trial is evaluation of safety and toxicity
- The **secondary endpoint** is evaluating antigen-specific anti-TNBC immune responses
  - PBMC and serum collected before, during, and after vaccination
  - Biopsy of skin at site intradermal injection given at time of 5th vaccination
  - Circulating tumor cell levels
  - Clinical follow up

Specific RAC Reviewer Comments

Dr. Hammarskjold-

Will there be any tests to specifically determine potential "auto-immune" reactions to such cells in vivo and/or in vitro?

Dr. Kiem-

Clarify follow-up after the last leukapheresis. Since a significant percentage of patients will survive long-term how often will they be tested / evaluated for any autoimmune abnormalities?

Assessment of Autoimmunity

- Medical history, review of systems, and physical examination performed regularly throughout the study
  - Clinical screening (week -6)
  - Weekly during the vaccination period
  - 2 weeks after the 6th vaccination
  - 1, 2 and 3 months after the 6th vaccination
  - During regularly scheduled medical oncology clinical follow up
- Laboratory testing for autoimmunity
  - ANA, RF, TSH, Anti-thyroglobulin Abs
  - Prior to vaccination (week -5)
  - 2 weeks after the 6th vaccination

Thank You