An observational study to assess T cell activation and the mechanism of immune system response in patients receiving trastuzumab for solid tumor treatment

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A. Objectives

a. To gain insight into the mechanism of trastuzumab action in cancer patients.
b. To assess whether T cell activation occurs in patients with solid tumors who are receiving trastuzumab therapy alone, or in combination with standard chemotherapy.

B. Background

Recent developments in cancer therapy utilize monoclonal antibodies which recognize tumor or cell-surface proteins. One such example is trastuzumab, a monoclonal antibody that recognizes a specific protein or "antigen" on the surface of breast epithelial cells. This antigen is known as the HER-2/neu growth factor receptor. Clinical trials have shown trastuzumab, in combination with chemotherapeutic agents, to be more effective than chemotherapy alone in the treatment of HER-2 positive advanced breast cancer (1,2). Additionally, new studies are addressing the role of trastuzumab in treating other solid epithelial tumors, i.e. advanced ovarian and prostate cancer (3).

The exact mechanism through which trastuzumab exerts its anti-tumor effect in the body is unknown. Multiple pathways may be involved, including blockade of cellular signaling, induction of programmed cell death, and direct antibody-mediated cell destruction or "cytotoxicity". More recently, data suggest that monoclonal antibody therapy may act to help specialized cells of the immune system, known as T cells, to recognize tumor antigen (4). Once primed and activated, T cells go on to initiate systemic immune responses in the body that destroy tumor cells.

In order for T cells to be activated, they must recognize and bind to a complex containing tumor derived peptides on the surface of antigen-presenting cells, or APCs. The context of the tumor peptide in the surface complex determines the type of immune response that ensues. For example, if the tumor peptide is presented by a class I molecule, a class I response occurs. A class 11 response is induced when T cells meet APC's carrying peptide restricted to a class 11 context. Different subpopulations of T cells, classified by the surface antigen markers CD8 and CD4, carry out class I and II responses, respectively. CD4-positive T cells carry out a class II antibody mediated immune response, and CD8-positive T cells mediate a class I systemic cytotoxic response. Both CD4- and CD8-mediated responses are required for effective tumor immunity (5).

Recent studies suggest that immune complexes are potent activators of both class I and II restricted antigen presentation, and thus have the potential to generate a combined CD4 and CD8 T cell response (4, 6). Passive administration of anti-tumor antibodies are likely to form immune complexes with tumor antigens such as HER-2, which may initiate a HER-2-specific class I and II responses.

To date, the hypothesis that a tumor-specific systemic T-cell response occurs in patients receiving monoclonal antibody therapy is untested. We propose to study whether or not T cell activation occurs in patients with solid tumors receiving HER-2/neu antigen should be amplified in blood samples taken from patients after monoclonal antibody treatment (normally given weekly doses for 6- 10 weeks).

T-cell activation can be measured by standard T-cell proliferation and cytokine induction assays in the laboratory (6). In addition specific binding of major histocompatibility complex tetramers which are constructed to bind to T cells expressing HER-2/neu will be used to detect and quantify the T cell response (7, 8). Also, we plan to measure trastuzurnab-specific antibody titers, as well as skin hypersensitivity test responses before and after treatment, in orderto ascertain whether HER

2-specificclass II responses are occurring in the patients. Previous work has shown that a HER-2 peptide induced skin hypersensitivity response >10mm 2 correlated with a peptide-specific peripheral blood T cell response (9).

Tumor-targeted activation of the immune system to requires a complex sequence of events, which likely begins with T cell activation. By examining this process in cancer patients receiving **T cell** activation in patients receiving trastuzurnab, we hope to better understand how monoclonal antibodies work to prevent tumor growth, in order to design more effective ways to treat and prevent cancer.

C. Eligibility Criteria

- Patients with HER-2-positive solid tumors (e.g. breast, ovarian, lung, uterine or prostate cancer) who are receiving trastuzumab and/or chemotherapy including but not limited to paclitaxel, docetaxel, flourouracil or estramustine.
- Patients who show no signs of myelosuppression, i.e. ANC>1000, ALC>400, Hb>8.0, plts>90K
- Patients who have not undergone chemotherapywithin the last 4 weeks.

Baseline bloods to assess HLA type, CBC, and to be banked.
Signed informed consent indicating full understanding of the risks and potential benefits of the research as outlined in the consent form.

D. Patient Entry And Procedure

a. Identification by primary physician of patients receiving trastuzumab therapy

Clinical information regarding the type and stage of solid tumor, as well as treatment history will be noted. Patients receiving trastuzumab alone or with chemotherapy will be consented.

b. Registration

Fax or deliver the completed Eligibility Criteria Form and signed Informed Consent to the Columbia Cancer Center Protocol Office, located on 6 Garden North Knuckle- room 435. Phone (212)305-8615, fax (212)305-3035. Send hard copy or faxed registration by intrahospital mail.

c. Required forms

Eligibility Criteria Form
Informed Consent

E. Study Procedures

a. Bloods

- Blood samples (less than 50 cc) will be collected at day 0, 21, and 42 of trastuzumab administration in patients with HER-2-positive advanced breast, ovarian, and prostate cancer.
- Blood will be drawn in purple -top and EDTA-tubes and sent to the laboratory of Dr. Raphael
- Clynes at P & S 8-5 10. The initial sample will be used for CBC, HLA typing, antibody titer, and T cell assays. All samples will be frozen and stored at -20'C in the laboratory of Dr. Clynes (P and S 8-510).

F. Skin tests

- Skin hypersenstivity tests for mumps, candida, trichophyton, and HER-2 peptides will be performed on days 0 and 42. A total of 0.01 cc antigen will be injected intradermally. The extent of induration will be recorded 48 hours later.

G. Required Data

Tests and Observations On study	Study Week		
	0	3	6
History	X		
Bloods	X	X	X
Skin tests	X		X

H. Statistical Considerations

This study attempts to detect an increase in HER-2 specific T cell responses and/or antibody titers in response to trastuzumab therapy. A number of in vitro assays will be attempted including serological assays (ELISA) for antibody titers and cell-based assays for T cell responses. We aim to detect a minimum 5-fold increase in antibody titers and similar increase in T cell frequency. Using a tetramer assay, researchers measuring T cell activation pre- and post- melanoma peptide vaccination found more than a 5 fold log 10 increase in peptide-specific T cells (8). We would anticipate performing assays on 7 evaluable subjects in each arm (Herceptin alone, and chemotherapy + Herceptin). This total of 7 patients per arm using a two sided Mest with a significance alpha level of 0.05 yields a 80% power to detect a 5-fold increase in T cell response (T cell frequency increases of 0. 1 to 0. 5%) in each group, assuming a standard deviation of 0.44.

I. References

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CONSENT

You are receiving herceptin, a monoclonal antibody therapy, and/or chemotherapy for your cancer. We are trying to understand exactly how herceptin works in the body and how it triggers the immune system to recognize and destroy tumor cells. Clinical researchers have developed ways to test what kind of immune response occurs in patients receiving herceptin. By studying your body's immune response, we may be able to design better, more effective ways to treat, and perhaps prevent cancer.

If you have chosen to participate in this study, you will have four tubes of blood (about 2 ounces total) drawn before you start treatment. Also, you will have a simple skin test done, the same one that doctors routinely use to test for TB exposure. In this case, part of the protein which herceptin recognizes will be injected under the skin of your forearm (about one drop).' You will need to return in 48 hours for a nurse to check for a skin reaction.

If your blood is not a certain type, we will not be able to measure your immune response with the tests that we have, and no further blood or skin tests will be done. If you have the desired blood type, three tubes of blood will be drawn when you come for treatment on week 3 and week 6. The skin test will be repeated on week 6. All of your blood samples will be frozen, and used only for experimental purposes.

STUDY BENEFITS

You may or may not benefit personally from this study. The benefits from participating in this study include helping to advance understanding of how herceptin works to fight cancer.

COSTS AND COMPENSATION

You will not receive any financial compensation for your participation in this study. The physician costs will be handled as per your insurance.

CONFIDENTIALITY

Information obtained during this study will be kept confidential. You will not be identified in any report or publication unless you sign a release. You consent to the publication of study results so long as the information is anonymous so that identification cannot be made. You should also be aware that authorized representatives of the National Cancer Institute (NCI) or the Food and Drug Administration (FDA) may examine your medical records related to this study but that there will be no breach of confidentiality.

PARTICIPATION IS VOLUNTARY

Your participation in this study is completely voluntary. You are not obliged in any way to participate. You may withdraw from this study at any point. Your decision to withdraw or not to participate will not affect your medical care at Columbia-Presbyterian Medical Center now or in the future. Signing this form does not waive any of your legal rights. We encourage you to ask questions regarding any aspect of the study that is not clear before you sign this consent form.

QUESTIONS

If you have any questions, please ask, and we will do our best to answer them. If you have additional questions in the future, you can contact the Principal Investigator, Dr. Rafael Clynes at 212-305-5289. If you have questions about your rights as a research participant, you may contact the Institutional Review Board at 212-305-5883.

PATIENT STATEMENT OF CONSENT

I have discussed this study with Dr. _____ to my satisfaction. I understand that my participation is voluntary and that I can withdraw from the study at any time. However, after the delivery of high-dose chemotherapy, marrow and peripheral blood cells must be infused. I have read the above and agree to enter this research study. Signing this form does not waive any of my legal rights.

I have been informed that if I believe that I have sustained injury as a result of participation in a research study, I may contact the Principal Investigator Dr. Rafael Clynes at 212-305-5289 or the Office of the Institutional Review Board at 212-305-5883, so that I can review the matter and identify the medical resources which may be available to me.

I understand that:

a. The Presbyterian Hospital will famish emergency medical care determined to be necessary by the medical staff of this hospital.

b. I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage.

c. No monetary compensation for wages lost as a result of injury will be paid to me by Columbia-Presbyterian Medical Center.

d. I will receive a copy of this consent form.

Date:

Patient's Signature: _____

Physician's Signature: _____

Witness' Signature: _____

The Institutional Review Board of the Columbia Presbyterian Medical Center has approved the solicitation of subjects to participate in this research proposal.