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14. ABSTRACT
Antibody-NKG2D ligand fusion protein targeted against the breast tumor antigen HER2. Using murine breast tumors designed to express the HER2 target antigen and the murine NKG2D ligand Rae-1β, we constructed an anti-HER2 IgG3-CH3-Rae-1β fusion protein by genetically fusing Rae-1β at the 3' end of CH3 domain of anti-HER2 IgG3 antibody. The purified Rae-1_ fusion proteins demonstrated ability to bind antigen on tumor cells and NKG2D on NK cells. The antibody-Rae-1_ fusion proteins showed increased cytotoxic activity directed against tumor targets.

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Breast, cancer, therapy, fusion protein

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ANTIBODY-NKG2D LIGAND (RAE-1BETA) FUSION PROTEIN FOR BREAST CANCER THERAPY

INTRODUCTION

NKG2D ligands are inducible stress response molecules expressed on virally infected and transformed cells.[1] NKG2D ligands activate the NKG2D receptor, a C type lectin-like receptor expressed on effector cells belonging to the innate and adaptive immune systems, and offer an effective link between innate and adaptive immunity necessary to mount potent anti-tumor response.[2] Over-expression of NKG2D ligands has led to tumor regression in multiple murine tumor models.[3,4] In contrast to observations derived from murine tumor models, the wide spread expression of these ligands on many human cancers does not generate the anticipated tumor-specific innate or adaptive response seen in mouse tumor models. One explanation for this is the shedding of these ligands into the blood stream and down-regulation of the NKG2D receptor on effector cells. This has the effect of both reducing the surface expression of these ligands on tumor cells while blunting the effectiveness of the receptor itself. Over-expression of NKG2D ligands on tumor cells effectively restores the balance of NK cell activation status in favor of stimulatory signals, provides a potent costimulatory signal to CD8+ T cells and can stimulate an effective anti-tumor response. Since most women who succumb to breast cancer harbor metastatic disease, direct transduction strategies effectively employed in murine experimental models to express NKG2D ligands will not be practical.

Our proposal seeks to combine NKG2D ligand, Rae-1β, with the targeting specificity of an anti-tumor antibody by producing antibody-Rae-1β fusion protein specific for the tumor antigen HER2.[5] The proposal wishes to employ the targeting flexibility of antibodies to direct localization of Rae-1β to the tumor site. We hypothesize that anti-HER2 antibody homing to HER2-positive tumor cells will deliver the NKG2D ligand, Rae-1β, to tumor cells. Local delivery of NKG2D ligand will effectively re-target NK cell cytotoxicity to tumor cells, leading to an enhanced innate response and priming of an adaptive response mediated by $CD8^+$ T cells with anti-tumor specificity.

The objective of this proposal is to develop and test antibody fusion proteins directed against known tumor associated antigens, e.g. HER2, fused to the NKG2D ligand, Rae-1β, of NK cells. I. Generation of an antibody-Rae-1β fusion protein. II. Investigation of the immune properties of anti-HER2 antibody-Rae-1β fusion protein *in vitro* and *in vivo*. III. Study of the antibody-Rae-1β fusion protein *in vivo* for ability to inhibit tumor growth and/or cause tumor regression in syngeneic animal tumor models.

BODY

<u>Specific Aim I:</u> Generation of an antibody-Rae-1β fusion protein.

Task 1. Construction and expression of anti-HER2 IgG3-C_H3-Rae-1β fusion protein (Months 1-2).

a. Construct anti-HER2 antibody-Rae-1β fusion gene.

b. Express anti-HER2 antibody-Rae-1β fusion gene.

Task 2. Production of anti-HER2 IgG3-C_H3-Rae-1β (Months 3-6).

a. Produce anti-HER2 antibody-Rae-1β fusion protein in milligram quantities.

b. Generate a murine mammary tumor cell EMT6-HER2 expressing Rae-1β (EMT6-HER2-Rae-1β).

Construction and purification of anti-HER2 IgG3-Rae-1β fusion proteins

We describe the construction and characterization of the Rae-1β fusion antibodies in which the extracellular domain without GPI linkage region of the Rae-1β molecule was fused by genetic engineering to the carboxy terminus of the heavy chain of an anti-HER2 IgG3 antibody. We opted to use the IgG3 backbone for the antibody molecule, since the extended hinge region of IgG3 would be expected to provide greater flexibility in folding to accommodate the presence of Rae-1β in the fusion antibody. IgG3 also exhibits Fc-mediated functions, such as complement activation and Fcγ binding. We chose the Rae-1β NKG2D ligand because NKG2D ligands overexpressed on tumor cells activate both the innate and adaptive arms of the immune responses.

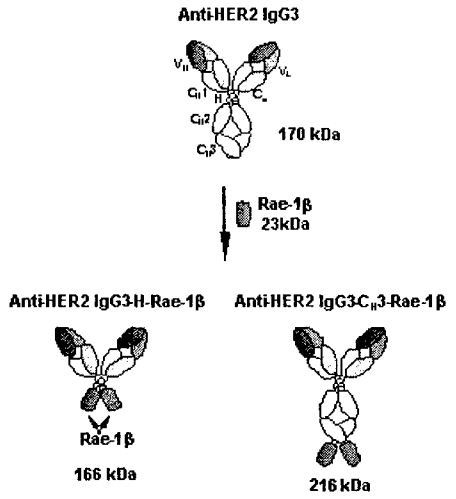

Fig. 1. Schematic diagram of the proposed anti-HER2 IgG3-Rae-1β fusion proteins.

The expression vectors for the human IgG3 heavy and κ light chains were previously constructed in the laboratory. To construct a fusion antibody between anti-HER2 IgG3 and Rae-1β, the extracellular domain of Rae-1β was cloned at the 3' end of the heavy chain constant region of anti-HER2 IgG3. Anti-HER2 IgG3-hinge-Rae-1β (H-Rae-1β) and anti-HER2 IgG3-C_H3-Rae-1β (C_H3-Rae-1β) fusion proteins have been generated to produce a more effective form of

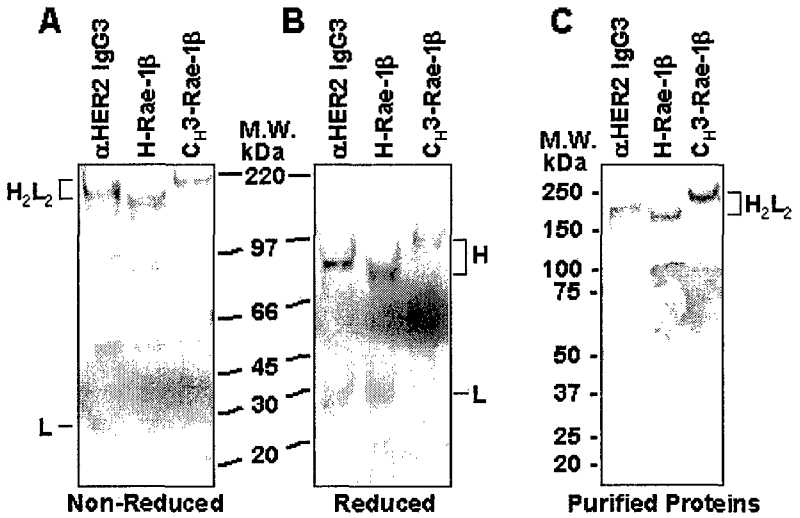

Fig. 2. Immunoprecipitation and SDS-PAGE analysis of anti-HER2 IgG3- Rae-1β fusion protein. The secreted IgG3-C_H3- Rae-1β fusion proteins biosynthetically labeled with [^{35}S] methionine was immunoprecipitated with Protein A and analyzed under non-reducing (A) and reducing (B) conditions. Rae-1β fusion proteins purified by affinity chromatography were analyzed under non-reducing conditions (C). Control anti-HER2 IgG3 (αHER2 IgG3) is included for comparison.

NKG2D ligand, Rae-1β, and to explore the possibility the antibody-Rae-1β fusion protein would target tumor expressing HER2 while retaining NK cell activating activity (Fig. 1). The anti-HER2 IgG3-Rae-1β genes were constructed and transfected into the murine P3X63Ag8.653

myeloma cell line. The anti-HER2 IgG3-Rae-1β fusion protein was purified using a Protein A column. An anti-HER2 IgG3-Rae-1β fusion protein of the expected molecular weight was secreted as the fully assembled H_2L_2 form (Fig. 2).

Specific Aim II: Investigation of the immune properties of anti-HER2 antibody-Rae-1β fusion protein *in vitro* and *in vivo*.

Task 3. Determination of tumor targeting ability and tissue biolocalization of Rae-1β fusion protein (Months 4-5).

a. Binding ability of anti-HER2 antibody-Rae-1β fusion protein to EMT6-HER2 tumor cells.
b. Binding ability of anti-HER2 antibody-Rae-1β fusion protein to NK cells.
c. Biodistribution and biolocalization of antibody-Rae-1β fusion protein in tumor bearing mouse.

Task 4. Analysis of biological functions of Rae-1β fusion protein *in vitro* (Months 6-7).

a. Determine *in vitro* cytotoxicity of syngeneic NK cells to EMT6-HER2-Rae-1β.
b. Determine *in vitro* cytotoxicity of syngeneic NK cells to either EMT6-HER2 with/without treatment of Rae-1β fusion protein.

Antigen binding ability of anti-HER2 IgG3-Rae-1β fusion proteins

Whether anti-HER2 IgG3-Rae-1β fusion proteins also retained the specific binding ability to HER2 antigens has been examined with tumor cell line expressing HER2 (such as EMT6-HER2, MC38-HER2, or CT26-HER2). Anti-HER2 antibody-Rae-1β fusion proteins have been tested for binding to murine tumor cell lines using anti-human IgG or anti-Rea-1β using flow cytometry (Fig.3). Bound anti-HER2 IgG3-Rae-1β fusion proteins to HER2 have been detected by anti-murine Rae-1β antibody conjugated with FITC. Both C_H3-Rae-1β and H-Rae-1β have

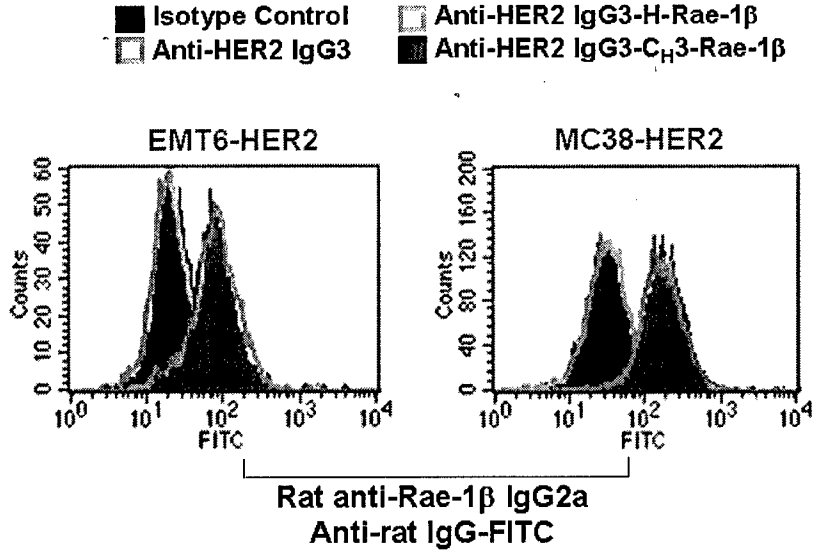

Fig. 3. Binding ability of anti-HER2 IgG3-Rae-1β fusion proteins to HER2 antigen. Both C_H3-Rae-1β (filled with red color) and H-Rae-1β (blue colored line) have been investigated to recognize HER2, but anti-HER2 IgG3 (green colored line) and isotype control (filled with purple color) did not have been detected.

recognized HER2, but anti-HER2 IgG3 and isotype control (anti-Dansyl IgG3) did not have been detected.

To investigate binding ability of the Rae-1β moiety in fusion proteins to NKG2D receptor using flow cytometry, anti-HER2 antibody-Rae-1β fusion proteins have been tested for binding to NK cells freshly isolated from C57BL6 or KY-2 cells (murine NK cell line) which express NKG2D (Fig. 4). Bound anti-HER2 IgG3-Rae-1β fusion proteins to NKG2D have been detected by anti-human IgG conjugated with FITC. Anti-HER2 IgG3-C_H3-Rae-1β showed stronger binding ability than anti-HER2 IgG3-H-Rae-1β on both NK cells. It might be the result of conformational difference between C_H3-Rae-1β and H-Rae-1β, and/or due to lack of Fc region in H-Rae-1β the detection antibody, anti-human IgG-FITC, might recognize H-Rae-1β less efficiently than C_H3-Rae-1β. However the control antibodies, anti-dansyl IgG3 and anti-HER2 IgG3, did not show any binding to NK cells. Bound anti-HER2 IgG3-Rae-1β fusion proteins to HER2 have been detected by anti-murine Rae-1β antibody conjugated with FITC. C_H3-Rae-1β and H-Rae-1β showed equivalent binding ability to tumor cells expressing HER2, while the control antibodies were not detected with anti-Rae-1β antibody-FITC.

These results demonstrate the anti-HER2 IgG3-Rae-1β fusion proteins will bind tumor cells and Rae-1β fusion proteins will bind NKG2D on NK cells through Rae-1β moiety. The NKG2D:Rae-1β interaction may stimulate NK cells and will cause tumor lysis by secreted perforin or granzyme B from the activated NK cells.

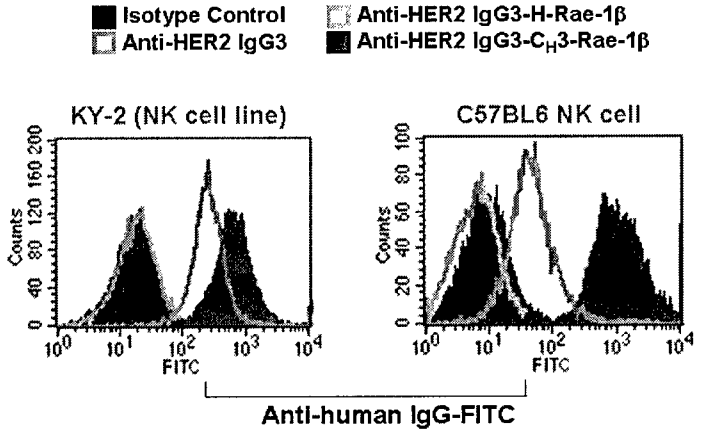

Fig. 4. Binding ability of anti-HER2 IgG3-Rae-1β fusion proteins to NKG2D. The Rae-1β moiety of anti-HER2 antibody-Rae-1β fusion proteins has been tested for binding to NKG2D on freshly isolated NK cells or murine NK cell line KY-2 cells. Both C_H3-Rae-1β (filled with red color) and H-Rae-1β (blue colored line) have been detected through the Rae-1β moiety, but anti-HER2 IgG3 (green colored line) and isotype control (filled with purple color) did not have been detected through the Rae-1β moiety.

Enhanced perforin production in KY-2 with anti-HER2 IgG3-Rae-1β fusion protein.

To evaluate the capacity of anti-HER2 IgG3-Rae-1β fusion protein to stimulate expression of perforin in NK cells, murine NK KY-2 cells activated with IL-2 (100U) have been stimulated in the presence of anti-HER2 IgG3-C_H3-Rae-1β fusion protein at the various concentrations (0.1 μg, 0.5 μg, or 2 μg) and controls: anti-HER2 IgG3 (2 μg) and isotype control (2 μg). Anti-HER2 IgG3-C_H3-Rae-1β fusion protein promote perforin expression in KY-2 cells in a dose-dependent manner (Fig.5). This result confirmed that the Rae-1β moiety of anti-HER2 IgG3-C_H3-Rae-1β fusion protein is functional.

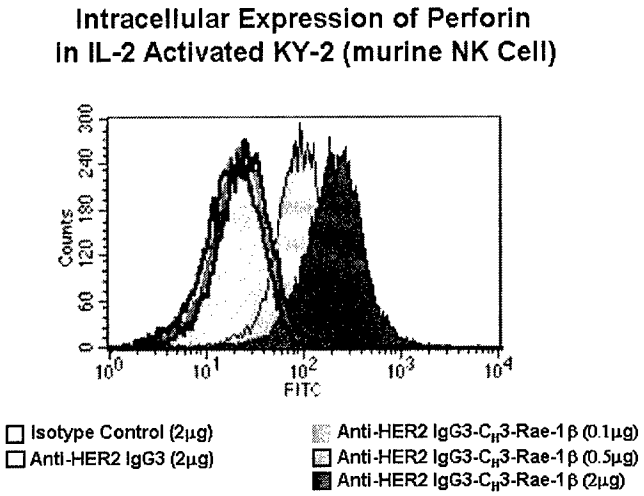

Fig. 5. Anti-HER2 IgG3-Rae-1β fusion protein-mediated enhancement of perforin production in KY-2 NK cells. Histograms demonstrate intracellular perforin expression of IL2 (100U)-stimulated KY-2 cell cultured in the presence of anti-HER2 IgG3-C_H3-Rae-1β fusion protein at the various concentrations (0.1 μg: filled with blue color, 0.5 μg: filled with orange color, 2 μg: filled with red color), anti-HER2 IgG3 (2 μg: blue colored line), and isotype control (2 μg: black colored line).

Augmented tumor-directed NK cell-mediated cytotoxicity by anti-HER2 IgG3-Rae-1β fusion protein.

To determine whether anti-HER2 IgG3-Rae-1β fusion proteins enhance the tumoricidal activity of NK cells, freshly isolated NK cells were cultured in the presence of anti-HER2 IgG3-Rae-1β fusion proteins (10 μg/well), anti-HER2 IgG3 (10 μg/well), or control anti-dansyl IgG3 (10 μg/well). After two days of stimulation, cytotoxic potential of NK cells toward the tumor cell line, MC38 expressing HER2 antigens (MC38-HER2), was evaluated in a 5-hr ^{51}Cr release assay (Fig. 6). The NK cell-mediated cytotoxicities have been done with EMT6-HER2 and CT26-HER2, but the results have been shown similar. Thus, the representative NK cytotoxicity has been presented.

Anti-HER2 IgG3 exhibited little tumor-directed cytotoxicity (20-40%) by NK cells, while anti-dansyl IgG3 did not show cytotoxicity (15-20%), (Fig. 6). Interestingly, whereas the H-Rae-1β fusion protein exhibited only little improvement of tumor-directed cytotoxicity (20-40%) by NK cells, the C_H3-Rae-1β fusion markedly enhanced NK cell-mediated killing activity (50-97%), (Fig. 6). The cytotoxic activity of the H-Rae-1β fusion protein was less potent than the C_H3-Rae-1β fusion protein perhaps due to the absence of an Fc region. These data illustrate that

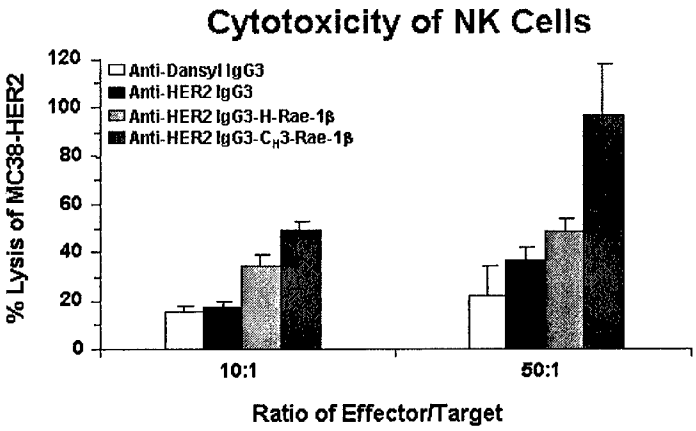

Fig 6. Enhancement of tumor-directed NK cell-mediated cytotoxicity by anti-HER2 IgG3-Rae-1β fusion protein. Freshly isolated NK cells were stimulated in the presence of anti-HER2 IgG3-Rae-1β fusion proteins (10 μg/well, C_H-Rae-1β; filled with red color, H-Rae-1β; filled with green color), anti-HER2 IgG3 (10 μg/well, filled with purple color), or control anti-dansyl IgG3 (10 μg/well, black line). After 2 days, NK were cocultured in round-bottom 96-well plates with the ^{51}Cr-labeled tumor cell lines MC38-HER2 at different E:T ratios. After 5 h of incubation, chromium release was measured. The results of three different donors are presented as mean ± SE of triplicate wells.

both the Rae-1β domain and Fc region of the fusion antibody play important roles in tumor-directed cytotoxicity mediated by NK cells.

Specific Aim III: Study of the antibody-Rae-1β fusion protein *in vivo* for ability to inhibit tumor growth and/or cause tumor regression in syngeneic animal tumor models.

Task 5. *In vivo* mechanistic investigation with anti-HER2/*neu* antibody-Rae-1β fusion protein. (Months 7-12).

a. Determine whether EMT6-HER2-Rae-1β cells are rejected in mice.
b. Investigate whether treatment of antibody-Rae-1β fusion slow or regress tumor growth in mice bearing EMT6-HER2.
c. Investigate anti-tumor activity of antibody-Rae-1β fusion in EMT6-HER2 bearing mice, in which either of NK, $CD4^+$, or $CD8^+$ cells is depleted.
d. To investigate whether NK cell-mediated tumor rejection promotes T cell immunity, mice that reject tumors will be rechallenged with either irrelevant tumors (CT26 or CT-26 HER2) or relevant tumors (EMT6, EMT6-HER2, or EMT6-HER2-Rae-1β).

We did not complete the Aim III yet, but future efforts in the laboratory will continue to focus on testing efficacy of the fusion proteins in vivo in tumor models. This will include in vivo targeting and tumor challenge experiments using the Rae-1β fusions using the CT26 and EMT6 breast cancer model to confirm the tumor regression experiment results and to determine the potential for engaging host immune responses. Finally we are actively pursuing the preclinical development of the Rae-1β fusion due to the efficacy in CT26-HER2 and EMT6-HER2 models.

KEY RESEARCH ACCOMPLISHMENTS

1. Anti-HER2 IgG3-Rae-1β fusion proteins of the expected molecular weight were secreted as the fully assembled H_2L_2 form.

2. Anti-HER2 IgG3-Rae-1β fusion proteins bound to HER2+ on tumor cells and Rae-1β fusion proteins recognized NKG2D on NK cells through Rae-1β moiety.

3. Anti-HER2 IgG3-C_H3-Rae-1β fusion protein promotes perforin expression in KY-2 in a dose-dependent manner.

4. The C_H3-Rae-1β fusion markedly enhanced NK cell-mediated killing, while the H-Rae-1β fusion protein exhibited only little improvement of tumor-directed cytotoxicity.

5. Both Rae-1β moiety and Fc region of the fusion antibody appear to play important roles in tumor-directed cytotoxicity mediated by NK cells.

REPORTABLE OUTCOMES: Patent Application

This results has been presented at Department of Defense Breast Cancer Research Program Meeting, The 4th Era of Hope in Philadelphia, Pennsylvania, June 8-11, 2005. Symposium 34 and Poster (P39-12): Appendix 1.

A provisional patent has been filed on June, 2005 (Appendix 2). This provisional patent covers an anti-tumor antibody and a receptor ligand fused into a single protein for use as an anti-cancer therapeutic. A non-limiting example includes a fusion protein of anti-HER2 antibody fused to the NKG2D ligand Rae-1β for a breast cancer therapeutic.

CONCLUSIONS

The purified Rae-1β fusion proteins demonstrated ability to bind antigen on tumor cells and NKG2D on NK cells. The antibody-Rae-1β fusion proteins showed increased cytotoxic activity directed against tumor targets.

The anti-HER2 antibody (Herceptin) is approved for the treatment of metastatic breast cancer. However, Herceptin is effective only in a small percent of patients whose tumors express HER2. Antibody-based cancer therapy is thought to lead to tumor destruction by activation of antibody dependent cytotoxicity (ADCC) and/or through direct effects on signaling by targeted receptors such as HER2. ADCC may be a major anti-cancer mechanism and it could be more effectively elicited in the presence of activated effector cells with increased cytolytic capacity derived through activation of a local innate immune response.

Furthermore we believe that an enhanced local innate response may lead to more efficient priming of an adaptive T cell mediated response. We have therefore used the targeting capabilities of an antibody to direct delivery of NKG2D ligand to the surface of tumor cells through the design and synthesis of an antibody-NKG2D ligand fusion protein. Local delivery and expression of NKG2D ligands on tumor cells effectively restores the balance of NK cell activation status in favor of stimulatory signals, provides a potent costimulatory signal to $CD8^+$ T cells and can stimulate an effective anti-tumor response.

Antibody-NKG2D ligand fusion proteins could potentially be used to treat multiple different types of malignancies by substituting other tumor antigenic specificities in the antibody domain (e.g. EGFR, CD20, PSMA, etc). Once this concept is validated in mouse models, the murine NKG2G ligand in the antibody fusion molecule could be replaced with human NKG2D ligands such the MHC class I-related chain A and B, and UL16 binding proteins (ULBP1, ULBP2, ULBP3, ULBP4) for testing in humansThis invention refers specifically to the targeting of NKG2D ligands to tumor cells using an antibody-NKG2D ligand fusion protein targeted against a specific tumor associated antigen, in this specific case, the breast tumor antigen HER2.

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APPENDICES

Appendices 1 and 2 have been attached.

Appendix 1

Agenda — June 11, 2005

5:15–6:45 p.m. **CONCURRENT SYMPOSIA SESSIONS VI**

Symposium 33 - ***Relationship between Biomarkers and Breast Cancer Pathology II*** — **Room 201A**

Co-Chairs: Dennis C. Sgroi and Patricia Haugen

Human Tissue Kallikreins as Biomarkers for Breast, Ovarian, and Other Malignancies
Eleftherios Diamondis

Novel Image Analysis to Link Sub-Nuclear Distribution of Proteins with Cell Phenotype in Breast Neoplasia
David W. Knowles

Cancer Specific Proliferating Cell Nuclear Antigen as a Novel Diagnostic Marker for the Detection of Breast Cancer
Derek Hoelz

Sandwich Test ELISA with SCFV Antibodies: An Alternative to an All-Time Favorite
Nathalie Scholler

Symposium 34 - ***Antibodies: Innate versus Engineered*** — **Room 201B**

Co-Chairs: Louis M. Weiner and Linda Vincent

Circulating Autoantibodies for Early Detection of Breast Cancer
Jean Latimer

Treatment of Breast Cancer with Antibodies against Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Death Receptors in Combination with Chemotherapy
Donald Buchsbaum

Inhibition of Breast Cancer Metastasis with Ligand Mimetic Antibodies from Cancer Patients
Brunhilde Felding-Habermann

Antibody-NKG2D Ligand (RAE-1BETA) Fusion Protein for Breast Cancer Therapy
Seung-Uon Shin

Cloning, Expression, and in Vitro Activity of a Pair of Truncated Bi-Specific Antibodies Targeting C1q to HER2
Xiaofeng Yang

Symposium 35 - ***Biological Function of High Risk Genes*** — **Room 201C**

Co-Chairs: Andrew K. Godwin and Mary Jo Vazquez

Effects of Estrogen, Pregnancy, and Therapeutic Drugs on Mammary Tumor Formation in BRCA1-Conditional Mutant Mice
Chuxia Deng

Genome Wide Analysis of Allelic Imbalance in Tumor Epithelium and Stroma in BRCA1- and BRCA2-Related Breast Cancers
Frank Weber

BRCA1-Deficient Mammary Tumors Have Distinct Tumorigenesis Processes and Chemotherapy Response
Christopher R. Smith

BRCA1 Interacts with Highly Conserved Components of the Transcription Elongator Complex
Craig B. Bennett

BRCA1 Directly Modulates Gene Expression Required for Estrogen Biosynthesis: A Possible Mechanism of Tissue-Specific Tumor Suppression
Yanfen Hu

Symposium 36 - ***DNA Damage and Repair II*** — **Room 204A**

Co-Chairs: Zhenkun Lou and Karin D. Noss

Investigating the Genetic Interaction of the Homologous Recombination Proteins: RAD51, BRCA2, and BLM
Teresa Marple

Involvement of Human MOF in ATM Function
Girdhar G. Sharma

Based on Selye's framework of Physiological Response to Stress and Lazarus and Folkman's Transactional Model of Stress, the specific aims of this study are to: (1) examine the association of objective and subjective breast cancer risk with immune responses; (2) examine the mediating role of psychological distress on the relationship between subjective breast cancer risk and immune responses; (3) determine the moderating role of dispositional optimism on the relationship between subjective breast cancer risk and psychological distress; and (4) assess the association between objective and subjective breast cancer risk in healthy women with (FH+) or without (FH-) family history of breast cancer in FDR.

For this cross-sectional study, a convenience sample of 94 healthy women (33 FH+, 61 FH-) completed self-report questionnaires for objective and subjective breast cancer risk, psychological distress and dispositional optimism and provided a blood sample. Objective breast cancer risk was calculated using the modified Gail model. NKCA and LAKCA were determined by a chromium-51 release cytotoxicity assay using K562 target cells.

Preliminary analyses indicated no association between objective and subjective breast cancer risk with NKCA and LAKCA (Aim 1), and no mediating role for psychological distress on the subjective breast cancer risk immune relationships (Aim 2). However, the moderating role of optimism on the relationship between subjective breast cancer and psychological distress was supported ($p = .013$) (Aim 3). In addition, objective and subjective breast cancer risk showed a positive significant correlation ($p=.003$) (Aim 4).

The results of these preliminary analyses indicate additional studies with larger samples of women having family history in FDR may help to advance the understanding of psychological-immune interactions in healthy women with varying degrees of breast cancer risk, and aid in developing better preventive strategies against breast cancer in the future.

The U.S. Army Medical Research and Materiel Command under W81XWH-04-1-0352 supported this work.

P39-12: ANTIBODY-NKG2D LIGAND (RAE-1BETA) FUSION PROTEIN FOR BREAST CANCER THERAPY

Seung-Uon Shin, Joseph D. Rosenblatt, Hyun-Mi Cho, and Khaled Tolba
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NKG2D ligands are inducible stress response molecules expressed on virally infected and transformed cells. NKG2D ligands activate the NKG2D receptor, a C type lectin-like receptor expressed on effector cells belonging to the innate and adaptive immune systems, and offer an effective link between innate and adaptive immunity necessary to mount potent anti-tumor response. Over expression of NKG2D ligands has led to tumor regression in multiple murine tumor models. In contrast to observations derived from murine tumor models, the wide spread expression of these ligands on many human cancers does not generate the anticipated tumor-specific innate or adaptive response seen in mouse tumor models. One explanation for this is the shedding of these ligands into the blood stream and down-regulation of the NKG2D receptor on effector cells. This has the effect of both reducing the surface expression of these ligands on tumor cells while blunting the effectiveness of the receptor itself. Over-expression of NKG2D ligands on tumor cells effectively restores the balance of NK cell activation status in favor of stimulatory signals, provides a potent costimulatory signal to CD8+ T cells and can stimulate an effective anti-tumor response. Since most women who succumb to breast cancer harbor metastatic disease, direct transduction strategies effectively employed in murine experimental models to express NKG2D ligands will not be practical.

We proposed the direct targeting of NKG2D ligands to tumor cells using an antibody-NKG2D ligand fusion protein targeted against the breast tumor antigen HER2. Using murine breast tumors designed to express the HER2 target antigen and the murine NKG2D ligand Rae-1beta, we constructed an anti-HER2 IgG3-CH3 Rae-1beta fusion protein by genetically fusing Rae-1beta at the 3' end of CH3 domain of anti-HER2 IgG3 antibody. We also constructed a retroviral vector of the full length of Rae-1beta (L-Rae-1beta-SDH) to generate a murine mammary tumor cell EMT6-HER2 expressing Rae-1beta. We have expressed a recombinant FLAG-tagged murine NKG2D soluble receptor for purposes of detection of Rae-1beta expression. We are now investigating the innate stimmulatory properties of anti-HER2 antibody-Rae-1beta fusion protein in vitro and in vivo. In addition ability of the Rae-1beta fusion protein to slow tumor growth in an animal tumor model and/or lead to both an innate and adaptive response will be investigated.

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Appendix 2

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APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
60/695,114	06/29/2005		100	7230-23	1		

CONFIRMATION NO. 3406

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AKERMAN SENTERFITT
P.O. BOX 3188
WEST PALM BEACH, FL 33402-3188

FILING RECEIPT

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Early Publication Request: No

** SMALL ENTITY **

Title

Antibody-NKG2D ligand fusion protein for cancer therapy

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