AD _____

GRANT NUMBER: DAMD17-98-1-8307

TITLE: Targeting Breast Cancer with Anti HER2/neu Diabodies

PRINCIPAL INVESTIGATOR: Louis M. Weiner, M.D.

CONTRACTING ORGANIZATION: Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

REPORT DATE: July 2000

TYPE OF REPORT: Annual

PREPARED FOR:
U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2000	Annual (22 Jun 99 - 21 Jun 00)

4. TITLE AND SUBTITLE
Targeting Breast Cancer with Anti HER2/neu Diabodies

5. FUNDING NUMBERS
DAMD17-98-1-8307

6. AUTHOR(S)
Louis Weiner, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

E-MAIL:
LM_Weiner@fccc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The objective of this proposal is to develop new therapeutic reagents for breast cancer. It is our hypothesis that improved diabody-based molecules with affinity for HER2/*neu* can be engineered and will prove to be effective vehicles for the RIT of breast cancer. The first Technical Objective (T.O.) focuses on the optimization of the production of the selected diabody and the identification of the optimal radionuclide and labeling strategy for diabody-based RIT. This T.O. also involves an investigation into the impact on diabody targeting and RIT of a variety of factors likely to be encountered in a clinical setting. These include the degree of antigen density, the route (i.v. bolus or continuous infusion) and frequency of administration, the presence of disseminated disease, and the effect of antigen expression on normal tissues. Completion of these experiments will set the stage for proceeding to the clinical evaluation of diabody-based targeting of breast cancer in our second Technical Objective. The clinical component of this proposal (to be initiated in year 3) will entail a Phase I radioimmunoimaging and radioimmunoguided surgery trial designed to elicit information on the dosimetry, specificity and tumor penetration properties of radiolabeled C6.5 diabody, and will assess the RIT potential of this molecule.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

MW Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

7/11/00

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION:

The objective of this proposal is to develop new therapeutic reagents for breast cancer. It is our hypothesis that improved diabody-based molecules with affinity for HER2/*neu* can be engineered and will prove to be effective vehicles for the RIT of breast cancer. The first Technical Objective (T.O.) focuses on the optimization of the production of the selected diabody and the identification of the optimal radionuclide and labeling strategy for diabody-based RIT. This T.O. also involves an investigation into the impact on diabody targeting and RIT of a variety of factors likely to be encountered in a clinical setting. These include the degree of antigen density, the route (i.v. bolus or continuous infusion) and frequency of administration, the presence of disseminated disease, and the effect of antigen expression on normal tissues. Completion of these experiments will set the stage for proceeding to the clinical evaluation of diabody-based targeting of breast cancer in our second Technical Objective. The clinical component of this proposal (to be initiated in year 3) will entail a Phase I radioimmunoimaging and radioimmunoguided surgery trial designed to elicit information on the dosimetry, specificity and tumor penetration properties of radiolabeled C6.5 diabody, and will assess the RIT potential of this molecule.

KEY RESEARCH ACCOMPLISHMENTS (YEAR 2):

- Development of a new preclinical targeting/therapy model.

We commonly employ *scid* mice bearing established s.c. human ovarian carcinoma SK-OV-3 tumors to assess the targeting properties of our anti-HER2/neu antibody-based molecules. However, as we noted in last year's progress report, the relative radiation insensitivity of the SK-OV-3 tumors limited the usefulness of the model for the evaluation of the C6.5 diabody-based radioimmunotherapy (RAIT). Accordingly, we acquired the human breast carcinoma MDA-MB-361 DYT2 subclone from Dr. Da Jun Yang of Georgetown University. This estrogen-dependent cell line grows rapidly *in vivo* (established tumors after approx. two weeks) and has previously proven to be an effective target in RAIT and immunodrug conjugate studies.

Our initial evaluation of this model entailed performing biodistribution studies to determine that radiometal-labeled C6.5 diabody was capable of targeting the MDA-MB-361 DYT2 tumors *in vivo*. The diabody was conjugated to the CHX-A" chelating agent and was subsequently labeled with In-111. Twenty micrograms were administered to each mouse by i.v. tail vein injection. Cohorts of 6 mice were euthanized after 4 or 24 hours and the localization of the labeled diabody in tumor and normal organs was determined. By four hours after injection, approximately 3 % of the injected dose localized per gram (%ID/g) of tumor and 2 %ID/ml remained in blood. At 24 hours after injection, the quantity in tumor had climbed to about 8 %ID/g and blood levels had dropped to 0.4 %ID/ml (tumor:blood ratio = 20:1). With the exception of the kidneys (6 %ID/g at 4 hours and 22 %ID/g at 24 hours), minimal quantities of the ^{111}In-CHX-A" C6.5 diabody were retained in all organs. This type of kidney retention is commonly observed when radiometal-conjugated antibody fragments are administered to mice. As

this effect has been reported to be inhibited by positively charged amino acids, we attempted to decrease the renal retention by administering an i.p. preinjection of 40 mg of L-lysine. This resulted in an approximately 50% decrease in the activity localized to the kidney with minimal alterations to the targeting of other tissues. Clearly, the immunoconjugate is capable of targeting the s.c. MDA-MB-361 DYT2 tumors.

- Determined the potential of radiolabeled C6.5 diabody to be utilized for the radioimmunodetection of breast cancer.

To determine the utility of employing the C6.5 diabody to deliver radioisotopes to tumors to facilitate radioimmunodetection (RAID), we performed gamma camera imaging studies with ^{111}In-labeled CHX-A"-C6.5 diabody and with ^{131}I-labeled C6.5 diabody. *Scid* mice bearing established MDA-MB-361 DYT2 tumors were given an i.v. injection of about 100 μg of ^{111}In- or ^{131}I-labeled C6.5 diabody. The mice were imaged 24 hours later on a gamma camera. The images performed with the ^{111}In-labeled diabody clearly revealed the s.c. tumors, but also displayed high renal retention. In contrast, the ^{131}I images failed to clearly display the tumors or any other organ. The combination of poor tumor imaging with the radioiodine labeled diabody and effective imaging with the radiometal labeled form indicated that selected catabolism was occuring leading to release of the radioiodine from the cell. This lead us to speculate that the HER2/*neu* target antigen may be rapidly internalized from the surface of the MDA-MB-361 DYT2 tumor cells (addressed below).

As the biodistribution studies described above indicated a preload of L-lysine reduces renal accumulation of radiometals that are conjugated to C6.5 diabody, we performed a gamma camera imaging study in the presence and absence of L-lysine to determine if the images could be improved. The L-lysine preload resulted in greater tumor-targeting specificity by four hours post injection and visibly reduced the quantity of In-111 in the kidneys at the 24 hour time point. However, as expected from the biodistribution studies, the preload did not completely eliminate the renal retention of the radiometal. Accordingly, we conclude that the use of radiometal-conjugated diabodies in clinical RAID trials will likely be associated with unacceptably high renal accumulation that could interfere with the detection of disease. Therefore, we will consider utilizing radioiodine-cconjugated diabody in forthcoming clinical trials.

- Characterized the internalization of the HER2/*neu* receptor on the MDA-MB-361 cell line.

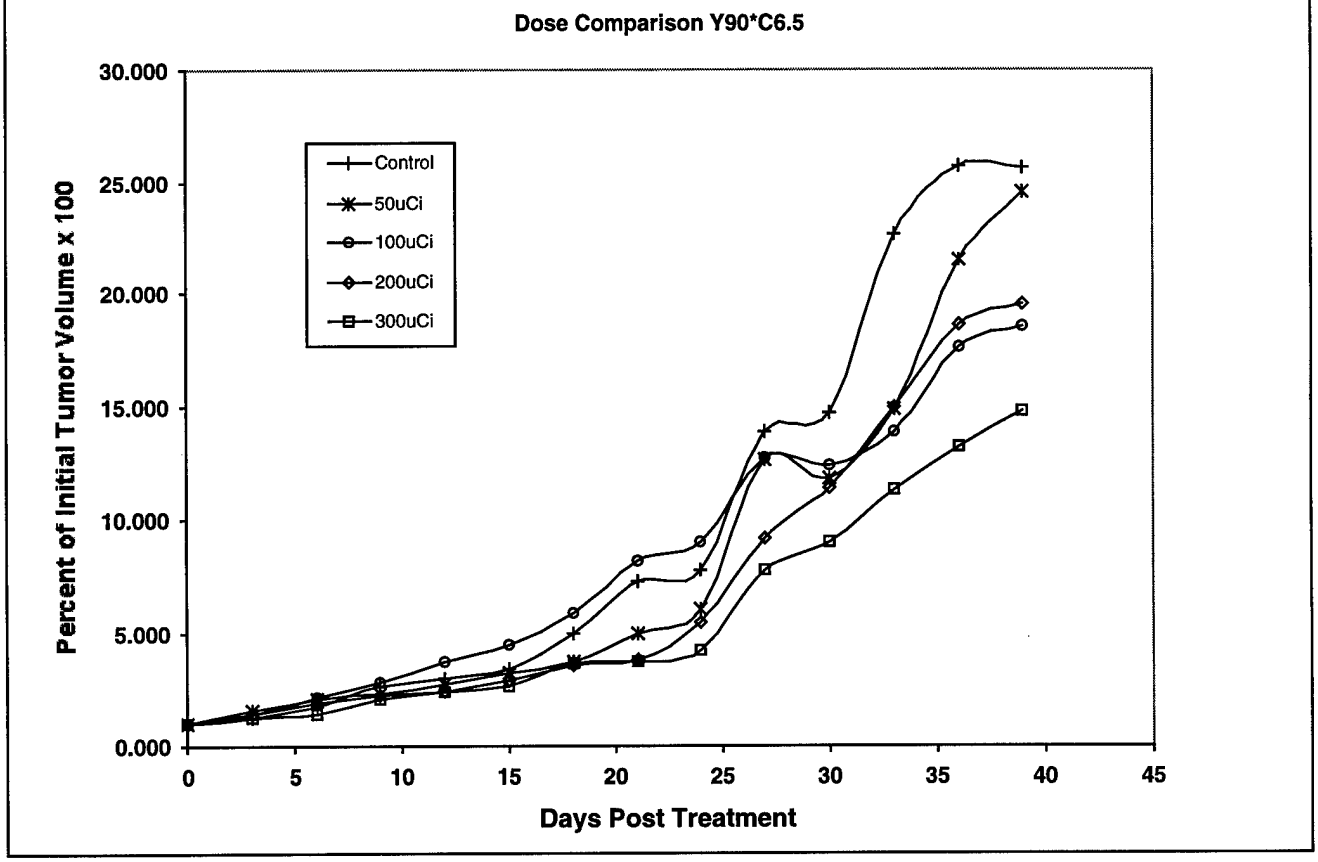
The disparate tumor-targeting results obtained with ^{131}I- and ^{111}In-conjugated diabody in the imaging studies suggested that the HER2/*neu* target antigen on the MDA-MB-361 DYT2 cells may be rapidly internalized. The internalization of radioiodinated proteins results in rapid dehalogenation and rapid elimination of radioiodine-tyrosine residues from the cell. In contrast, when radiometal labeled proteins are internalized and catabolized, the metals are retained in the cell. An *in vitro* internalization study

performed with ^{111}In-CHX-A" labeled C6.5 diabody indicated that the MDA-MB-361 DYT2 cells rapidly internalized the diabody after it was bound. These results were contrary to slow internalization we previously observed when the diabody was incubated with the human ovarian carcinoma cell line SK-OV-3. This suggests that differences exist between the HER2/*neu* receptor on the two cell lines. Rapid internalization can decrease the efficacy of using ^{131}I-C6.5 diabody for the proposed RAID and RIGS applications in the upcoming clinical trial. Accordingly, we will be expanding the *in vitro* internalization studies to examine other HER2/*neu* overexpressing cell lines (e.g., SK-BR-3, BT-474, etc.) and to include the 4D5 (Herceptin - fast internalizing) and 741F8 (slow internalizing) MAbs as controls.

- Compared the therapeutic efficacy of ^{90}Y-labeled-CHX-A"-C6.5 diabody in nude mice bearing established SK-OV-3 tumors and MDA-MB-361 DYT2 tumors.

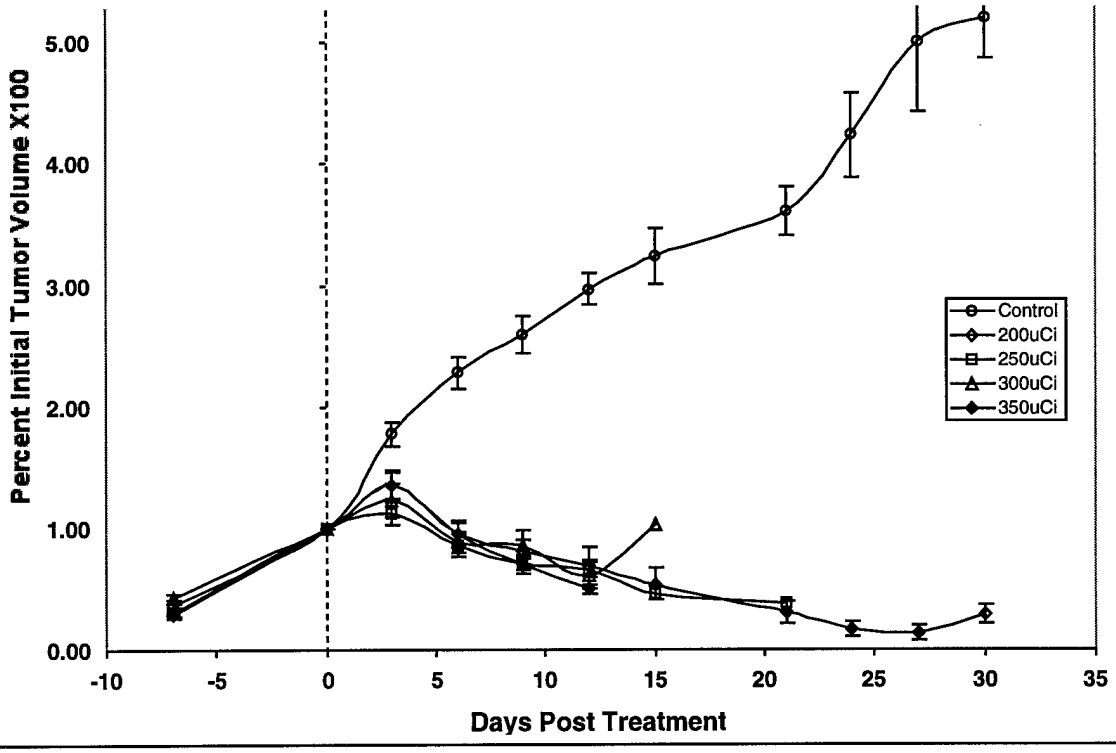
Preclinical RAIT studies were performed in *scid* mice bearing established s.c. SK-OV-3 or MDA-MB-361 DYT2 tumors on their abdomen. Therapy of the mice bearing the SK-OV-3 tumors resulted in a reduction in the rate of tumor growth as compared to that observed in untreated control mice (Figure 1). However, even at the highest dose of 300 μCi, tumor growth progressed.

Figure 1. 90Y-CHX-A" labeled C6.5 diabody RAIT in nude mice bearing s.c. SK-OV-3 tumors. Results are presented as percent of initial tumor volume. SEMs are indicated, n=10 mice per treatment group.



A similar treatment therapy study performed in mice bearing the MDA-MB-361 DYT2 tumors revealed significantly different results. All of the treatment doses lead to dramatic reductions in tumor volumes, with some complete responders. However, the therapy in this tumor model exhibited a greater degree of toxicity than did similar doses in the mice bearing SK-OV-3 tumors. All of the doses above 200 μCi were 100% fatal and 80% of the mice treated with the 200 μCi dose did not survive the therapy. To identify the MTD and therapeutic efficacy in this model, we have recently initiated a follow up therapy trial examining lower doses (90 - 200 μCi) of ^{90}Y-labeled-CHX-A"-C6.5 diabody in older (larger) mice. We believe that the differences in therapeutic efficacy and toxicity of ^{90}Y-labeled-CHX-A"-C6.5 diabody in the two models is related to the ultimate fate of the diabody that binds to tumor cells. If it is internalized, the isotope persists in the tumor until it eventually disintegrates causing DNA damage to both the tumor and (due to the small size of the mouse) proximal tissue. However, if the diabody is not internalized it can dissociate from the HER2/neu target antigen, diffuse out of the tumor and be eliminated via the kidneys prior to the therapeutic (and toxic) disintegration event.

Figure 2. 90Y-CHX-A" labeled C6.5 diabody RAIT in nude mice bearing s.c. MDA-MB-361 DYT2 tumors. Results are presented as percent of initial tumor volume. SEMs are indicated, n=10 mice per treatment group.



PUBLICATIONS THAT EMANATED FROM THIS GRANT:

- Manuscripts:

Adams, G.P., Schier, R., McCall, A.M., Crawford, R.S., Wolf, E.J., Weiner, L.M. and Marks, J.D. Prolonged *in vivo* tumor retention of a human diabody targeting the extracellular domain of human HER2/*neu*. British J. Cancer, 77:1405-1412, 1998.

Adams, G.P., Shaller, C.C., Chappel, L. Wu, C., Horak, E.M., Simmons, H.H., Litwin, S., Marks, JD., Weiner, L.M. and Brechbiel, M.W. Delivery of the alpha-emitting radioisotope Bi-213 to tumors via single-chain and diabody molecules. Nucl. Med. Biol. (in press).

Nielsen, U.B., Adams, G.P., Weiner, L.M. and Marks, J.D.. Targeting of bivalent anti-HER2/*neu* diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. (Submitted)

- Abstracts:

Nielsen, U.B., Adams, G.P., Weiner, L.M., and Marks, J.D. Bivalent diabodies versus high affinity scFv antibody fragments for tumor cell targeting. IBC's 9th Annual International Conference on Antibody Engineering (Coronado, CA), 1998.

Adams, G.P., Shaller, C.C., Chappel, L. Wu, C., Horak, E.M., Simmons, H.H., Marks, JD., Weiner, L.M. and Brechbiel, M.W. Delivery alpha-emitting radioisotopes to tumors via single-chain Fv and diabody molecules. Proc. Amer. Assoc. Cancer Res., 40:354,1999.

Nielsen, U.B., Adams, G.P., Weiner, L.M. and Marks, J.D. Targeting of bivalent anti-HER2/neu diabody antibody fragments to tumor cells is independent of intrinsic antibody affinity. Proc. Amer. Assoc. Cancer Res., 41:289, 2000.