AD_____

GRANT NUMBER: DAMD17-94-J-4176

TITLE: Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies

PRINCIPAL INVESTIGATOR: Carolyn Wasserheit, M.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10010-2598

REPORT DATE: September 1996

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1996	Annual (1 Sep 95 - 31 Aug 96)

4. TITLE AND SUBTITLE
Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies

5. FUNDING NUMBERS
DAMD17-94-J-4176

6. AUTHOR(S)
Carolyn Wasserheit, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York University Medical Center
New York, New York 10010-2598

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, 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*

Over the last year, we have opened a clinical trial using In-111 MX-DTPA huBrE-3 in patients with metastatic breast cancer. Three patients have been studied with no significant toxicity. Data available on two of the patients reveal that 22 of 26 known sites of metastasis were identified by scans and two previously unknown sites were identified. Plasma T½ clearance averaged 45.7±28.1 hours. Whole body doses extrapolated to Y-90 huBrE-3 averaged 2.55±0.39 rads/mCi with tumor doses averaging 95.5±8.3 rads/mCi. The degree of immunogenicity on repeated serum sampling was negligible. The results obtained support the further evaluation of Y-90 huBrE-3 as a potential therapeutic agent.

In the laboratory, experiments have been conducted in the nude mouse, human tumor xenograft model. A synergistic anti-tumor effect was observed in mice treated with a combination of topotecan and 90-Y MX-DTPA BrE-3 with complete eradication of tumor in the treated mice. We plan to continue these preclinical studies and hope to conduct a clinical trial using combination radioimmunoconjugate and topotecan in patients with advanced breast cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast cancer, radioimmunotherapy, monoclonal antibody	20
	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 US 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.

____ 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.

CW
__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).

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

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

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

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

Carolyn Wasserheit 9/3/96
PI - Signature Date

Table of Contents

Introduction

The following is a progress report for Grant No. DAMD17-94-J-4176 entitled, "Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies" for the period of September 1, 1995 to August 31, 1996.

The overall goal of the project is to develop effective therapy for breast cancer using radioimmunoconjugates. As noted previously, the humanized version of BrE-3 (huBrE-3) designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH) has become available to us. The purpose of the design was to diminish the immunogenicity of the frameworks while strictly maintaining the antigen binding affinity. This required that all amino acid interactions that might affect the conformation of the antigen binding surfaces be left intact. In compliance with this goal, all the framework amino acids have been mutated from murine to human identities except for those amino acids judged to be important for antigen binding. All of these "murine" residues which have been preserved have either inwardly pointing side chains or make contacts with the opposite antibody chain. Therefore, their side chains should not be available for binding and internalization by B-cells and, therefore, should not be immunogenic. Eight of these residues make CDR contacts; the consequences of replacing these "murine" amino acids which are important for antigen binding cannot be predicted.

Another potential strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. As noted in my previous progress report, at NYU, a model drug that we have helped develop is the topoisomerase-1 inhibitor topotecan (topo). Topoisomerase-1(topo-1) is a unique target for cancer chemotherapy. It is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. Drug interaction with this enzyme converts topo-1 into a "cellular poison" and results in progressive cell death.

We have conducted a Phase I study utilizing a novel schedule for administration of topo, under sponsorship of CTEP. In this study topo was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We have determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to conventional (daily x 5) administration schedule. We have also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens including taxol) and renal cancer (1). We are currently performing a phase I study evaluating 3 hour paclitaxel and 14 day continuous-infusion topotecan to evaluate the toxicity and response in patients with advanced cancer.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (2-6). It has been postulated that the synergism between the topo-1 inhibitors and

ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (7). Ionizing radiation sensitizes cells to topo-1 inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (4). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (5), or concurrently with continuous application (8, 9). Findings of synergism between topo and ionizing radiation in experimental models of lung cancer (3) have led to an ongoing clinical trial of combined external beam radiation therapy and topo in patients with mediastinal cancer, primarily lung cancer.

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index. The potential for increased toxicity of the two modalities is also present. Although this has not occurred consistently in experimental models of radioimmunotherapy and radiosensitizers (8), it has been observed with 5-bromodeoxyuridine (10) and with hypoxic cytotoxins (11). Clinically, increased toxicity has been seen in the more radiosensitive organs within the radiation port when radiosensitizers are used (12). For instance, increased pulmonary toxicity has been observed in patients undergoing lung irradiation with radiosensitizer administration (9). It should be noted, however, that topo-1 inhibition is not equivalent mechanistically to such radiosensitizers and these data are of unknown importance to the studies proposed here.

As previously reported, we have conducted experiments in the mouse model and demonstrated the feasibility of administering topotecan as a continous infusion. We also demonstrated an antitumor effect. As described below we have initiated experiments using continuous infusion topotecan and radioimmunoconjugate. The data generated thus far support the use of combination therapy for future clinical trials.

Body

Over the last year, we have initiated a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer (previously submitted to you). The objectives of this study are:

1) To assess the toxicity and efficacy of using a humanized ^{111}Indium labeled monoclonal antibody to localize tumor in patients with advanced breast cancer. To utilize nuclear medicine scanning to assess the ability of the monoclonal antibody to image sites of known disease in patients with advanced breast cancer.

2) To study the pharmacokinetics of this radiolabeled monoclonal antibody to develop dosimetry estimates to assess its potential as a radioimmunotherapeutic agent.

3) To assess the development of anti antibody response to administration of this antibody and to determine the nature of this response.

4) To assess expression of the BrE-3 antigen in human breast tumors by retrospective study of the pathology specimens.

Validation of sterility, apyrogenicity, and preservation of immunoreactivity were performed in September and early October 1995. To date, we have submitted the names of 70 patients for immunohistochemistry screening. Thirty-six of those patients have had tissue available and 25 patients have been positive (≥25% cell staining) and are eligible. We studied two patients in November and December 1995 respectively. They each received 2 mg of MX-DTPA huBrE-3 labeled with about 5mCi of Indium ^{111} plus 48 mg of nonconjugated BrE-3 intravenously over one hour. The antibody infusions were well tolerated. No allergic or toxic side effects were observed. Patients underwent serial whole body counting, gamma camera imaging, plasma and urine sampling over one week in order to assess pharmacokinetics, radiation dose and tumor localization. Imaging was quite promising in both patients. We imaged 22/26 (84.6%) known bone, liver, and lung lesions and identified two sites previously unsuspected (lymph node, bone). Blood and urine pharmacokinetics were also measured (Table 1, Appendix). Blood half-lives have been faster than the average blood half-lives observed in our patients studied with the murine antibody. In our first patient in whom antigen-antibody complex averaged 15.6% of the circulating radioactivity after antibody administration, a single compartment model gave a good fit to the data. A shorter plasma half life and smaller AUC was associated with higher circulating antigen. In our second patient, in whom the antigen-antibody complex averaged only 2.9% of the circulating radioactivity, a two-compartment model gave the best fit for the data. Radiation dose estimates (using standard MIRD formalism) have been made for normal organ and tumor (Table 2, Appendix). Liver uptake is somewhat higher in the patients receiving the humanized BrE-3 than average hepatic uptake of the murine antibody. Immunogenicity has now been studied out to 3 months in our patients. Qualitative analysis of serum incubated with either radioiodinated huBrE-3 or Indium-111 labeled hu BrE-3 demonstrates that compared to baseline serum, there are trace amounts of antibody-antibody formation at 5 weeks and 3 months after antibody infusion. Since these anti-humanized antibodies react equally with the murine BrE-3, we believe that "HAHA" represents an idiotypic response.

In August 1996 we studied an additional patient. She had no demonstrable toxicity to antibody infusion. We are currently in the process of evaluating tumor localization, radiation dose estimates, and pharmacokinetics. We are planning to study another eligible patient within the next month.

Thus far, the results suggest that it is possible to administer therapeutic doses of radioimmunoconjugates to patients. The relatively low immunogenicity may allow for repeated administration. Within the next year, we plan to initiate a phase I radioimmunotherapy study using Y-90 MX DTPA huBrE-3. The study will involve both escalation of the amount of radioactivity and the number of administration in cohorts of

three patients each. Based on promising preclinical mouse experiments described below, we hope to initiate a phase I clinical trial with combination Y-90 MX DTPA huBrE-3 and continuous infusion topotecan.

Over the past year we expanded upon our initial observations demonstrating significant anti-tumor efficacy of continuous infusion topotecan in the mouse model and have performed experiments using combination topotecan infusion and Y-90 MX DTPA BrE-3.

In the fall of 1995, we performed biodistribution experiments in PLCR female mice. Twelve mice were given an intraperitoneal injection of 50 micrograms (0.1 cc) of murine BrE-3 containing 200 uCi of Y-90. Three mice each were sacrificed at 6 hours, 24 hours, 48 hours, and 72 hours post injection. The graph in Figure 1 (Appendix) outlines the % injected dose of 90-Y per gram of tissue for each organ. The majority of 90-Y activity was in liver, kidney, and bone at 48 hours post injection.

In late 1995, early 1996 we began combination studies in the mouse model. In the first experiment we used 7 groups of 3 Swiss nude mice each. Each mouse received a subcutaneous implantation (into left flank) of an 8 mm^3 chunk of the human mammary carcinoma MX-1 (approximately 1 x 10^7 cells). Fourteen days later, the mice were randomly assigned to a treatment arm, and, except for the control mice, had insertion of an Alzet pump containing either saline alone or topotecan at varying concentrations. The pumps allow for a continuous infusion of topotecan for 7 days. At day 8, the pumps were removed and replaced with new pumps containing the same amount of topotecan (saline for group 2) as the first pump for each group. The second pumps were removed on day 14. Thus, the 5 groups of mice that were randomized to topotecan, received the drug as a 14 day infusion. After the mice were anesthetized with IP Avertin, the pumps were implanted by a small incision under the skin in the right flank. After the placement of the pump, the skin was stapled and the wound was disinfected with betadine. In addition, groups 2, and 4-7 received an intraperitoneal injection of 50 micrograms of murine MX-DTPA BrE-3 labeled with 180 uCi of 90-Yttrium on day 0 (14 days post tumor implantation). The groups were randomly assigned as follows: (Appendix, Figure 2)

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=3)	none	0	0
2 (n=3)	yes	0	180uCi
3 (n=3)	yes	2mg/m^2	0
4 (n=3)	yes	0.2mg/m^2	180uCi
5 (n=3)	yes	0.5mg/m^2	180uCi
6 (n=3)	yes	1.0mg/m^2	180uCi
7 (n=3)	yes	2mg/m^2	180uCi

The mice were observed for 3 weeks post therapy. As demonstrated in Figure 2, the control mice had continued tumor growth over 21 days, as expected. The groups treated with 90-Y-MX-DTPA-BrE-3 monoclonal antibody or with topotecan alone had a small decrease in tumor growth relative to control. The groups receiving combination therapy had a much greater decrease in the growth rate of the tumor. The greatest effect was noted in group 6 when topotecan was given at 1.0 mg/m^2 per 7 days. At the higher dose of topotecan (2.0 mg/m2 over 7 days) in combination with 90-Y-BrE-3, all the mice died, presumably from drug toxicity.

In April 1996, we performed another experiment using greater numbers of mice per group. Again, Swiss nude mice were implanted with MX-1 tumors fourteen days prior to starting therapy as described above. Two groups of mice received topotecan infusion for 14 days starting day 0 via Alzet pump as described above. For this and all subsequent experiments the dose of topotecan used was 1.0 mg/m^2 over 7 days as this was the most efficacious in the previous experiment. The dose of murine 90-Y-MX-DTPA BrE-3 used in this experiment was between 180-185 uCi in 50 microgram of total protein (0.1cc). This was given as a bolus IP injection on day 0. The mice were randomly assigned to the following groups:

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=5)	no	0	0
2 (n=7)	yes	1.0mg/m^2	0
3 (n=7)	yes	0	180uCi
4 (n=7)	yes	1.0mg/m^2	185uCi

As noted in Figure 3 (Appendix), the control group had continued tumor growth. The group of mice treated with 90-Y-BrE-3 or topotecan alone had a decrease rate of growth relative to the control. The combined therapy group had a dramatic decrease in tumor weight which persisted for the duration of the experiment (3 weeks). Thus, we demonstrated in this experiment that the tumor burden decreased from baseline in the combined therapy group.

We then performed a confirmatory and slightly different experiment in the Swiss nude mice. In this experiment the mice were implanted with MX-1 tumor as described previously. The tumors were allowed to grow for 21 days before randomization for therapy. Alzet pumps were implanted into the mice on day 21 as described above. Two groups of mice received 200 uCi of 90-Y-BrE-3, 21 days after tumor implantation. Two groups of mice received a 14 day infusion of topotecan also beginning 21 days post tumor implantation. The groups were randomized as follows:

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=6)	no	0	0
2 (n=7)	yes	1 mg/m^2	0
3 (n=7)	yes	0	200uCi
4 (n=7)	yes	1 mg/m^2	200uCi

As demonstrated in Figure 4 (Appendix), the mice treated with combination topotecan and 90-Y-BrE-3 had complete disappearance of tumor xenograft for up to 50 days post tumor implantation. The mice in groups 2-4 will continue to be observed until tumor progression develops.

The mice experiments conducted thus far provide promising evidence for the synergistic effect of combination topotecan and murine 90-Y-Mx-DTPABrE-3 monoclonal antibody in the mouse human tumor xenograft model. In the next several months we plan to conduct additional experiments which will compare the aforementioned combination therapy with 90-Y tagged to a nonspecific antibody, and with the humanized form of BrE-3. In this manner we will be better able to extrapolate the data obtained in mouse model to phase I clinical trials where we plan to use the humanized BrE-3. We also want to document that the specificity of BrE-3 to the tumor is adding to the antitumor effect, thus 90-Y will be tagged to a nonspecific antibody and the antitumor effect will be compared to that of 90-Y-BrE-3. We also plan to initiate in vitro experiments using MCF-7 and other breast cancer cell lines in an attempt to determine the mechanism of action of tumor cell kill with combination topotecan and radioimmunoconjugates.

Conclusion

In conclusion, over the last year we have initiated a radioimaging study of ^{111}In-Mx-DTPA-huBrE-3 in patients with metastatic breast cancer. We have treated 3 patients thus far. Preliminary results suggest that a therapeutic trial of 90-Y-Mx-DTPA huBrE-3 is feasible and repeated doses can be administered. In addition we have performed several experiments in the mouse model with combination radioimmunotherapy and continuous infusion topotecan. We have preliminary data demonstrating that the combination therapy eradicated the MX-1 implanted tumors in nude mice. Future studies in the mouse model will involve long term observation in the treated mice and possible repeat administration of combination therapy if tumor regrowth occurs. Toxicity studies will also be performed. In this manner, we hope to develop a phase I study using combined radioimmunotherapy and continuous infusion topotecan which will be of greatest benefit for women with metastatic breast cancer.

References

1. Hochster, H., L. Liebes, J. Speyer, J. Sorich, B. Taubes, R. Oratz, et al., Phase I trial of low dose continuous topotecan infusion (7-21 days) in patients with cancer; an active and well-tolerated regimen. J Clinical Oncology, 1993. **12**: p. 553-559.

2. Mattern, M., G. Hofmann, F. McCabe and R. Johnson, Synergistic cell killing by ionizing radiation and topoisomerase I inhibitor topotecan (SK&F 104864). Cancer Res, 1991. **51**: p. 5813-5816.

3. Chang, A., C. Gu, P. Keng and S. Sobel. Topotecan and etoposide inhibit radiation repair differently than potential lethal damage repair or sublethal damage repair. in Proc Annu Meet Am Assoc Cancer Res. 1992.

4. Del-Bino, G., S. Bruno, P. Yi and Z. Darzynkiewicz, *Apoptotic cell death triggered by camptothecin or teniposide.* The cell cycle specificity and effects of ioinzing radiation. Cell Prolif, 1992. **25**: p. 537-548.

5. Kim, J., S. Kim, A. Kolozsvary and M. Khil, Potentiation of radiation response in human carcinoma cells in vitro and murine fibrosarcoma in vivo by topotecan, an inhibitor of DNA topoisomerase I. Int J Radiat Oncol Biol Phys, 1992. **22**: p. 515-518.

6. Boscia, R., T. Korbut, S. Holden, G. Ara and B. Teicher, Interaction of topoisomerase I inhibitors with radiation in cis-diamminedichloroplatinum(II)-sensitive and -resistant cells in vitro and in the FSAIIC fibrosarcoma in vivo. Int J Cancer, 1993. **53**: p. 118-123.

7. Mattern, M., G. Hofmann, F. McCabe and R. Johnson. Synergistic cell killing by ionizing radiation and topoisomerase I inhibitor SK and F 104864. in Proc Annu Meet Am Assoc Cancer Res. 1990.

8. Santos, O., K. Pant, E. Blank and R. Ceriani, %-Iododeoxyuridine increases the efficacy of the radioimmunotherapy of human tumors growing in nude mice. J Nucl Med, 1992. **33**: p. 1530-1534.

9. DeGreve, J., F. Warson, D. Deleu and G. Storme, Fatal pulmonary toxicity by the association of radiotherapy and medroxyprogesterone acetate. Cancer, 1985. **56**: p. 2434-2436.

10. Morstyn, G., R. Miller, A. Russo and J. Mitchell, Iodine-131 conjugated antibody cell kill enhanced by bromodeoxyuridine. Rad Onc Biol Phys, 1984. : p. 1437-1440.

11. Wilder, R., V. Langmuir, H. Mendonca, M. Goris and S. Knox, Local hyperthermia and SR 4233 enhance the antitumor effects of radioimmunotherapy in nude mice with human colonic adenocarcinoma xenografts. Cancer Res, 1993. **53**: p. 3022-3027.

12. Kinsella, T., A. Russo, J. Mitchell, J. Collins, J. Rowland, D. Wright, et al., A Phase I study of intravenous iododeoxyuridine as a clinical radiosensitizer. Int J Radiat Oncol Biol Phys, 1985. **11**: p. 1941-1946.

APPENDIX

Appendix to Progress Report

Table 1: Blood pharmacokinetics

	Dose administered	T1/2 blood	AUC
01HBRDK	4.12 mCi	24.05 ± 4.5 hrs	1.91 ± 0.05 mCi •hrs
02HBRRL	2.99 mCi	α=23.3 ± 258.5 hrs β=100.7± 793.8 hrs	6.32± 1.54 mCi•hrs

Table 2: Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3 (rads/mCi)

Indium-111

	01HBRDK	02HBRRL	average	S.D.
kidneys	1.99	2.73	2.36	0.52
liver	5.91	2.71	4.31	2.26
lung	1.01	0.81	0.91	0.14
ovaries	0.51	0.46	0.49	0.04
red marrow*	0.5	0.45	0.48	0.04
red marrow†	0.93	1.43	1.18	0.35
spleen	3.46	2.08	2.77	0.98
urinary bladder	0.75	0.37	0.56	0.27
whole body	0.621	0.48	0.55	0.10

Yttrium-90

	01HBRDK	02HBRRL	average	S.D.
kidneys	14.9	27.6	21.25	8.98
liver	46.9	19.3	33.10	19.52
lung	5.85	5.99	5.92	0.10
ovaries	1.5	1.45	1.48	0.04
red marrow*	2.02	1.9	1.96	0.08
red marrow†	10.6	21.8	15.05	6.29
spleen	37.4	19.5	28.45	12.66
urinary bladder	1.81	1.07	1.44	0.52
whole body	2.82	2.27	2.55	0.39

*based on blood

†based on regions of interest

Figure 1

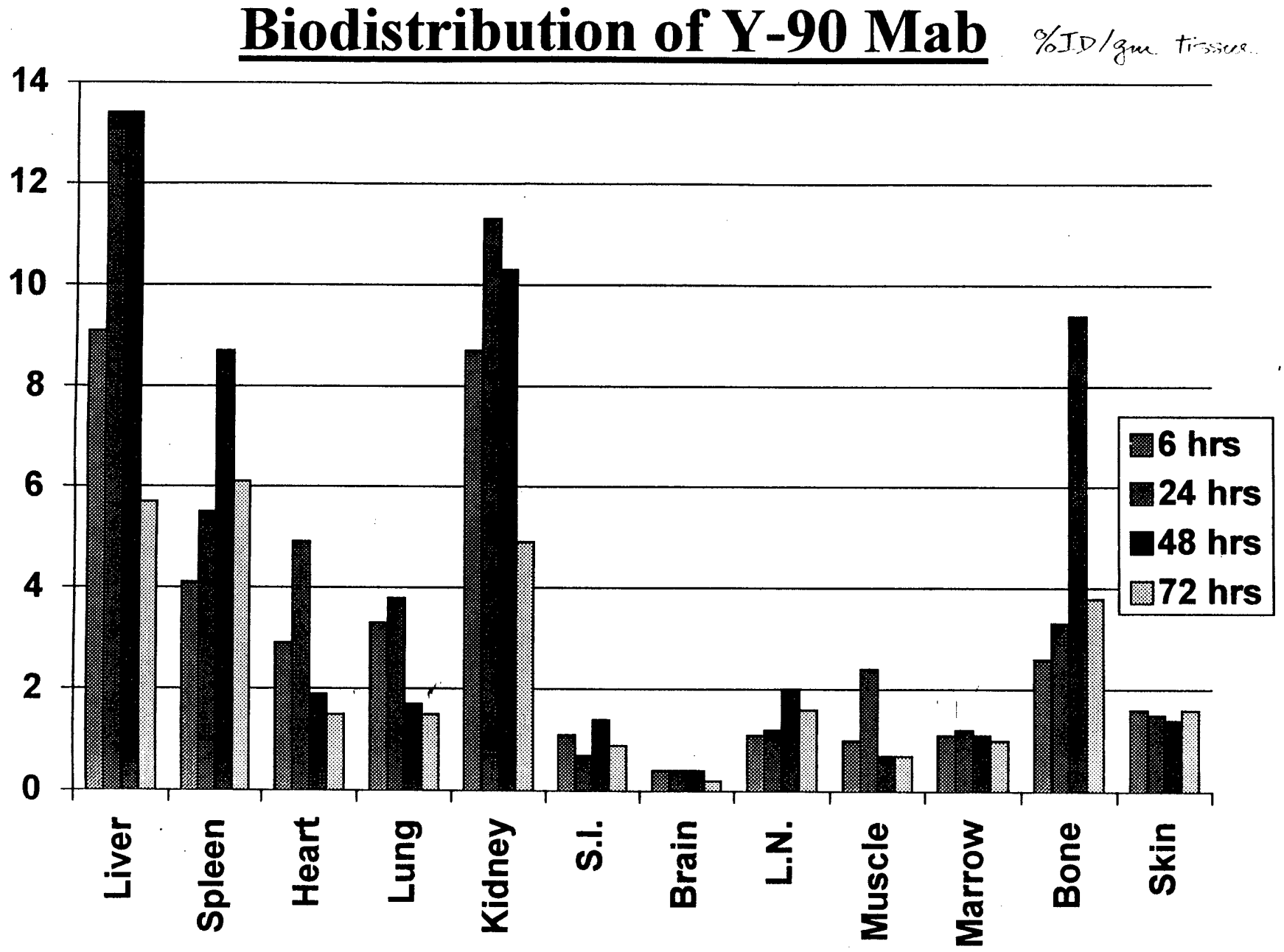

Biodistribution of Y-90 Mab
%ID/gm tissue
14
12
10
8
6
4
2
0
6 hrs
24 hrs
48 hrs
72 hrs
Liver
Spleen
Heart
Lung
Kidney
S.I.
Brain
L.N.
Muscle
Marrow
Bone
Skin

Figure 2

Topotecan and ^{90}Y Bre-3 MAb on Tumor Growth Results: Tumor Volume 3/15/96

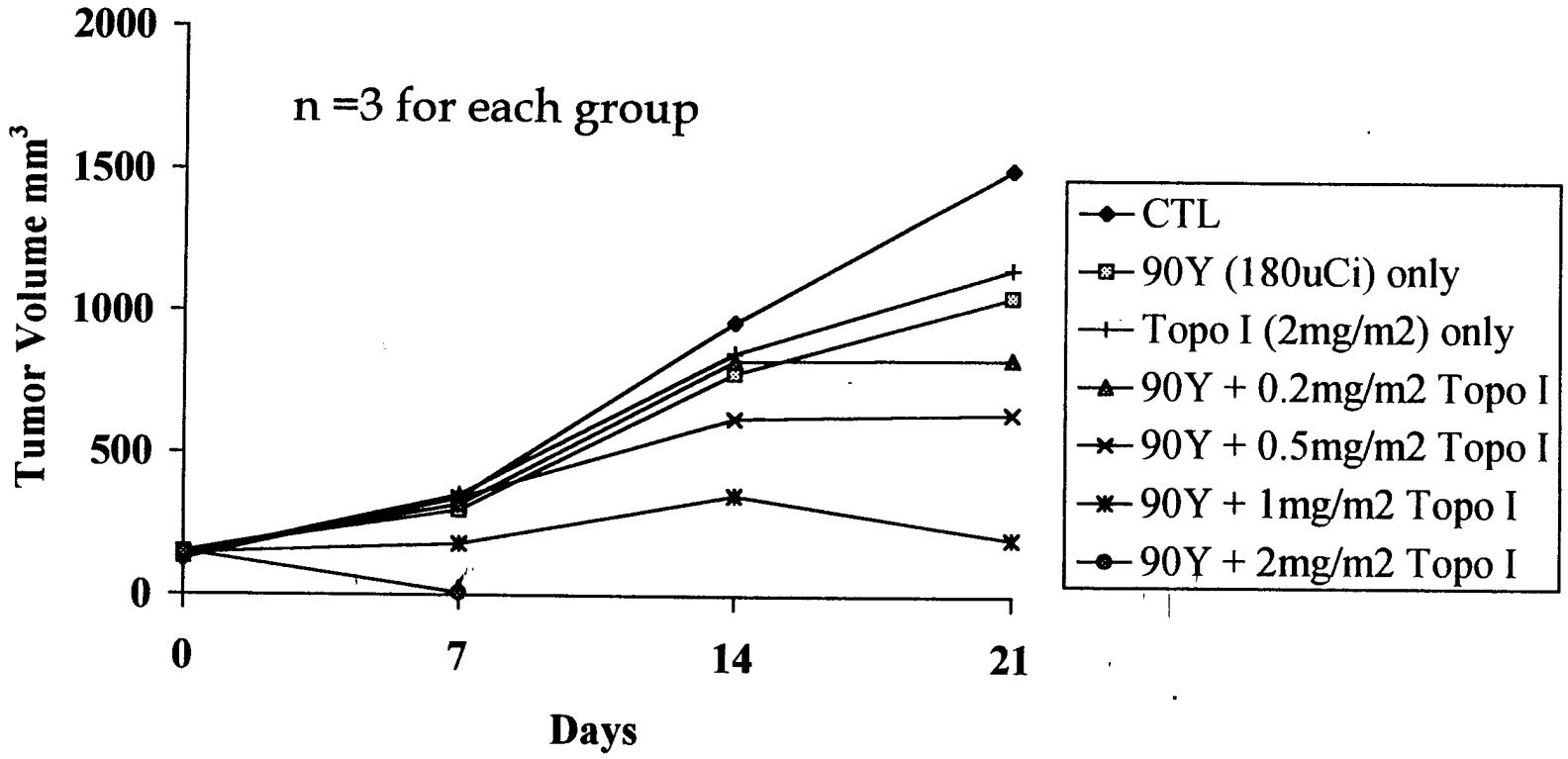


Figure 3

Effect of Topotecan and Bre-3 MAb 4/20/96 Results: Tumor Weights

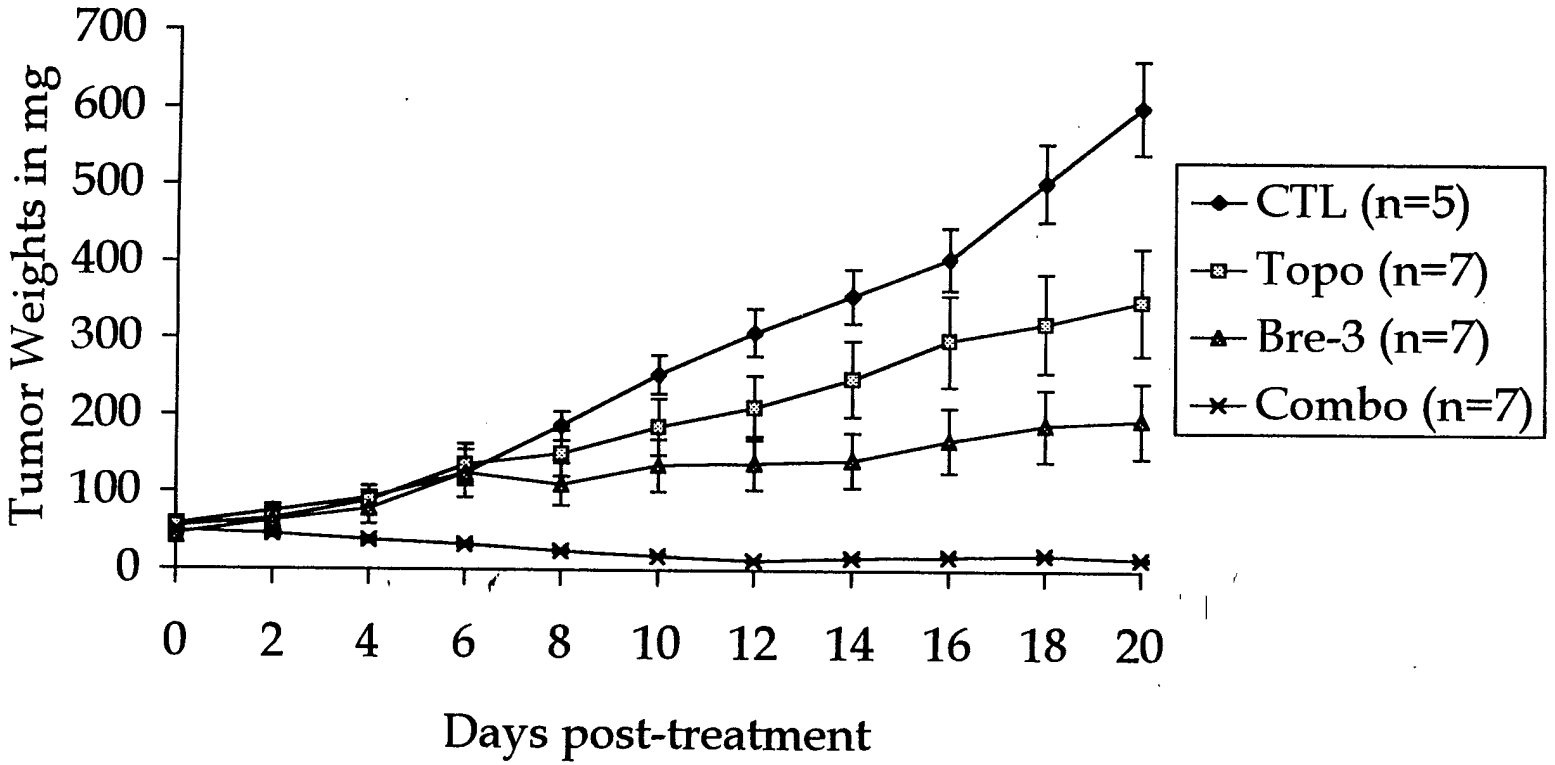
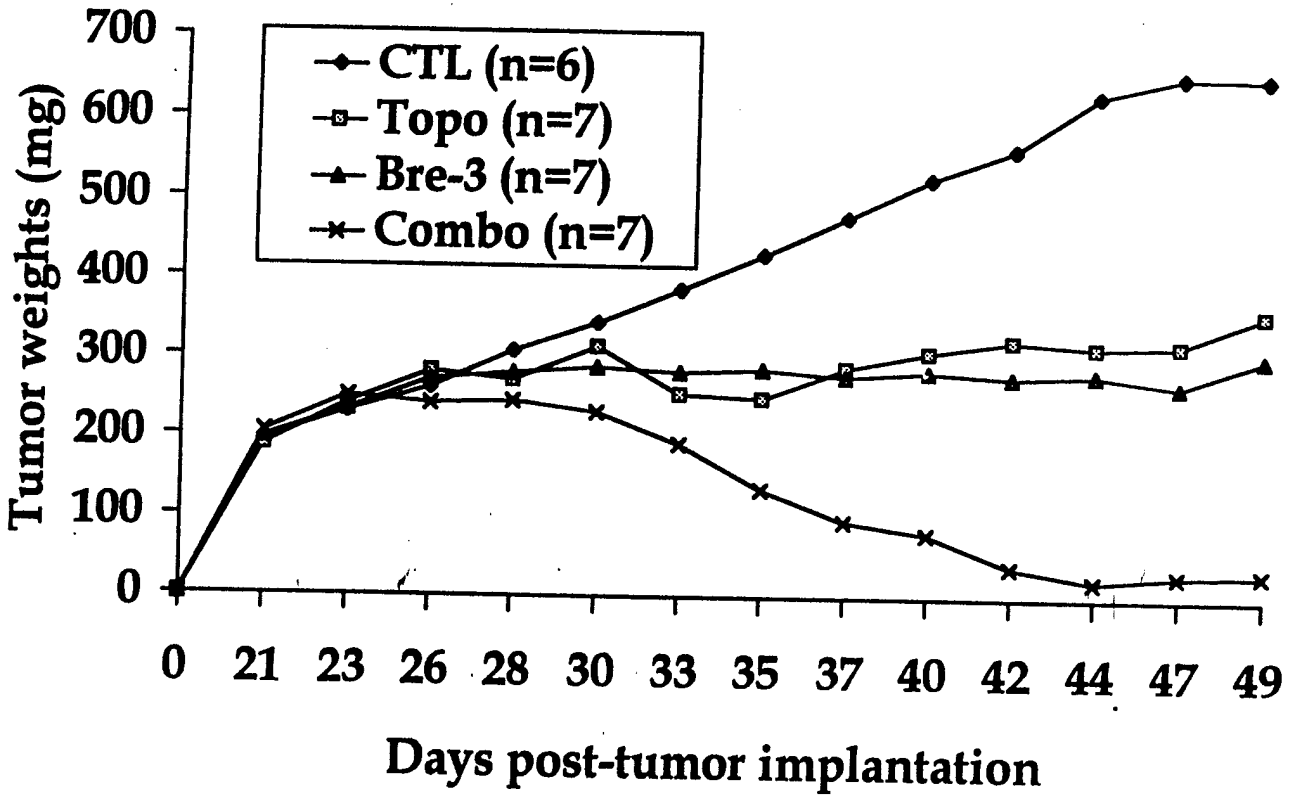


Figure 4

Effect of Topotecan (1mg/m^2) and Bre-3 MAb (200*u*Ci) 6/11/96

Results: Tumor Weights



LOCALIZATION OF IN-111 MX-DTPA HUMANIZED BRE-3 MAB IN PATIENTS WITH ADVANCED BREAST CANCER.

EL Kramer[1], L Liebes[1], C Wasserheit[1], E Blank[3], M Noz[1], D Dosik[1], H Mizrachi[1], T Kim[1], D Fry[1], A Zabalegui[1], J Sanger[1], R Ceriani[3], J Peterson[3], P Furmanski[2]. [1]New York University Med. Ctr./Bellevue Hosp. Ctr., and [2] Dept. of Biology, New York University, New York, NY; [3]Cancer Research Fund of Contra Costa, Walnut Creek, CA.

To evaluate MX-DTPA humanized BrE-3v1 monoclonal antibody (huBrE-3) as a potential radiotherapeutic immunoconjugate in breast cancer, In-111 MX-DTPA huBrE-3(2 mg, 185 MBq) was administered i.v. with 48 mg of nonconjugated huBrE-3 to patients with metastatic breast cancer. Serial whole body counting, gamma camera imaging, plasma, and urine sampling were performed to assess pharmacokinetics, radiation dose, and tumor localization. Scan results were compared to sites of tumor documented by conventional modalities. Pharmacokinetic modeling of plasma radioactivity and radiolabeled antibody was performed. Radiation dose was estimated using standard MIRD formalism. Two patients with 26 metastatic sites total have been studied so far. No toxicity or allergic reactions have been observed. 22/26 (84.6%) sites were identified on scans including liver, lung and bone metastases. Two previously unknown sites are suspected. Plasma T1/2 clearances for radiolabeled antibody averaged 45.7 ±28.1 hrs. AUC averaged 1.55± 1.02 %ID·hrs. A shorter plasma T1/2 and smaller AUC were associated with higher circulating antigen. Percent radioactivity excreted in urine averaged 71±22% in the first 48 hours. Calculated whole body dose for In-111 was 0.17 mGy/MBq. Percent ID/g estimated from serial imaging was 0.0004% in a measurable bone tumor. Extrapolating In-111 biodistribution to Y-90 biodistribution and radiation dosimetry, we estimate a dose to this tumor of 20mGy/MBq administered Y-90-huBrE-3 with a 0.42 mGy/MBq dose to marrow.

These early results demonstrate favorable pharmacokinetics and suggest that Y-90 huBrE-3 may be an active radioimmunotherapeutic agent. Immunogenicity studies and further patient studies are proceeding.
(Supported by NIH RO1 CA61455)

Published in the Journal of Nuclear Medicine, Volume 37, No. 5, 1996, p. 169.

INITIAL STUDIES IN PATIENTS WITH ADVANCED BREAST CANCER USING IN-111 MX-DTPA HUMANIZED BRE-3 (HUBRE-3) MONOCLONAL ANTIBODY. C. Wasserheit[1], E.L. Kramer[1], L. Liebes[1], M. Noz[1], A. Zabalegui,[1] E.Rios[2], D. Fry[1], E. Blank[3], J.Sanger[1], R. Ceriani[3], J. Peterson[3], and P. Furmanski[2]. [1]NYU Med. Ctr./ Bellevue Hosp. Ctr/ Kaplan Cancer Ctr and [2]Dept. of Biology, NYU, New York, NY 10016. [3]Cancer Research Fund of Contra Costa, Walnut Creek, CA 94596

To evaluate radiolabeled MX-DTPA huBrE-3 as a radioimmunotherapeutic agent in breast cancer, preliminary imaging, pharmacokinetic, and dosimetry studies with In-111 MX-DTPA huBrE-3 have been performed in patients with metastatic breast cancer expressing BrE-3 antigen. To date two patients have been studied. Patients received 2 mg MX-DTPA huBrE-3 labeled with 3-4 mCi In-111 combined with 48 mg of nonconjugated huBrE-3 over an hour. Serial blood samples, gamma camera imaging, whole body counting, and continuous urine collection were performed over one week. Abnormal sites of uptake on scan were compared to known lesions documented by conventional methods. Pharmacokinetic modeling of plasma samples which were subjected to HPLC was performed. Radiation dose estimates for normal organs and measurable tumors were made using the standard MIRD formalism. Immunogenicity of the huBrE-3 was evaluated up to 3 months after administration.

No significant toxicity or allergic reactions were observed. Twenty-two of 26 known sites of disease were imaged on planar and or SPECT scans. Two previously unsuspected sites of disease were seen and confirmed. Overall sensitivity was 86%. In one patient, mono-exponential modeling yielded a plasma T1/2 of 24.05 ±4.5 hrs and in the second a bi-exponential model gave an $T1/2_{alpha}$=23.3±258.5 hrs and $T1/2_{beta}$= 100.7 ±793.8 hrs. AUC's were 1.91 and 6.32 mCi·hrs, respectively. Average whole body dose for In-111 huBrE-3 was 0.55±0.10 rads/mCi. Whole body dose extrapolated to Y-90 huBrE-3 averaged 2.55±0.39 rads/mCi. Tumor doses averaged 95.5±8.3 rads/mCi. Serum samples at 5 weeks and 3 months after huBrE-3 administration showed only trace amounts of reactivity with both huBrE-3 and murine BrE-3.

The excellent tumor localization, favorable pharmacokinetics, and initial radiation dose estimates support the further evaluation of radiolabeled MX-DTPA huBrE-3 as a potential therapeutic agent. Although slightly immunogenic, the levels observed should not preclude repeated administrations. Further studies are anticipated.

To be presented at the poster session at the Sixth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer; October 10-12, 1996, Princeton, NJ.