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PRINCIPAL INVESTIGATOR(S): Carolyn Wasserheit, M.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10016

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Over the last year, we have worked to obtain a humanized version of the BrE-3 monoclonal antibody to have available for clinical trial. The humanized BrE-3 is now approved by the FDA and we are ready to start a radioimaging trial of ^{111}In MX-DTPA huBrE-3 in patients with metastatic breast cancer. In the laboratory we are using the mouse model to conduct experiments combining chemotherapy with radioimmunotherapy. We have performed studies evaluating the feasibility and efficacy of a continuous infusion of the topoisomerase 1 inhibitor, topotecan, in mice. We have demonstrated an antitumor effect of topotecan. Topotecan was successfully administered by continuous infusion in Alzet pumps. We are combining topotecan with IL-1 and exploring toxicity and efficacy. Our next set of experiments will incorporate radioimmunotherapy into this system.

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C Wasserheit 9/4/95

PI - Signature Date

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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, 1994 to August 1, 1994. I have previously notified you of some changes in the original proposal which are detailed below. The changes are directed toward improving the potential efficacy of radioimmunotherapy in breast cancer. The changes, however, have resulted in delay in the initiation of the clinical trials because we are awaiting the availability of the humanized version of the radioimmunoconjugate.

The overall goal of the project is to develop effective therapy for breast cancer using radioimmunoconjugates. In our initial proposal we planned to conduct a phase I study of murine 90 Yttrium MX-DTPA BrE-3 in women with metastatic breast cancer. Based on previous preliminary results, doses of ^{90}Y MX-DTPA of 12.25 mCi/m^2 in one patient resulted in significant hematologic toxicity, the development of HAMA and no antitumor effect. Over the last year we have been exploring methods to deliver radioimmunotherapy more effectively. One potential method is to be able to deliver repeated doses of radioimmunotherapy. A humanized version of the antibody would significantly reduce the immunogenicity of the monoclonal antibody and potentially allow for dose fractionation. Recently, humanization of both the constant and variable portion of the BrE-3 antibody has been accomplished with preservation of the CDR's of the antibody in their murine form, maintaining the affinity of the antibody. The humanized version of BrE-3 (huBrE-3) has been designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH). 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 in 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.

In spite of these remaining "murine" residues, the VL and VH frameworks of the humanized antibody are, respectively, 93% and 90% identical to the corresponding human frameworks (kII and HIIIc). This similarity to the human frameworks exceeds that of many human antibodies for their own consensus. The affinity of huBrE-3 for the BrE-3 antigen exceeds that of the murine antibody by three-fold. HuBrE-3 antibody stains human breast tumors in histological paraffin-embedded sections, clearly discriminating between normal and transformed cells.

Another potential strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. 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. Many effective chemotherapy drugs (e.g. doxorubicin and the other anthracyclines, podophyllotoxins, anthracene drugs) act on topoisomerase-2, an enzyme involved in double strand DNA unwinding and breakage. Furthermore, many of these same drugs are cross-resistant and are susceptible to resistance mediated by the Multi-Drug Resistance phenotype. Topo-1, however, which inhibits the resealing step of single strands breaks of DNA, is inhibited by camptothecin (CPT), a natural product from the plant *Camptotheca acuminata,* and its analogs. This compound has been part of the Chinese pharmacopeia as a plant extract for many years. The plant's active component, CPT converted into a sodium salt, was tested in the clinic as an anticancer drug in the early 1970's but abandoned because of unpredictable toxicity. Now it is recognized that the salt exists as carboxylated inactive form of CPT. More recently, interest in CPT has been stimulated by the awareness that its mechanism of action involves interaction with topoisomerase-I through formation of a "cleavable complex" involving a DNA:topo-1:drug configuration. With the development of newer analogs that are potentially more effective and less toxic than the parent drug, the opportunity exists to investigate this novel class of compounds in human cancer therapy and its mechanism of action.

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 proposed this study based on our observations that prolonged subcutaneous administration of 9-AC suspension to mice bearing human tumor xenografts, resulted in unprecedented anti-tumor activity, with little or no toxicity (1). To date we have treated 43 patients in a phase I study of dose escalating topo, reaching a 21 day duration at 0.7 mg/m2/day. 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 (2). This schedule deserves further investigation in the clinic and we now have plans to combine this schedule with radioimmunotherapy in phase I studies.

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* (3-7) 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 (8). Ionizing radiation sensitizes cells to topo-1

inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (5). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (6), or concurrently with continuous application (9,10). Findings of synergism between topo and ionizing radiation in experimental models of lung cancer (4) 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 (9), it has been observed with 5-bromodeoxyuridine(11)and with hypoxic cytotoxins (12). Clinically, increased toxicity has been seen in the more radiosensitive organs within the radiation port when radiosensitizers are used (13). For instance, increased pulmonary toxicity has been observed in patients undergoing lung irradiation with radiosensitizer administration(10). 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.

Body

Over the last year, huBrE-3 has been produced in large quantity by the Cancer Research Fund of Contra Costa. Purification of one gram of huBrE-3 has been completed. Necessary safety testing has been successfully performed. This includes testing for sterility and mycoplasma contamination. The antibody has also been conjugated by Dr. Quadri at M.D, Anderson. The chelate is identical to that utilized with the murine monoclonal antibody, MX-DTPA, produced by IDEC pharmaceuticals. Successful conjugation has been completed and an IND was submitted to the FDA several months ago for which we recently gained approval.

In preparation for clinical trials using huBrE-3 at NYU, we have obtained New York University approval to conduct a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer. 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.

The protocol is submitted as appendix 1. It is similar to our initial radiolocalization trial with the murine antibody. We will study up to 15 patients with advanced breast cancer. Patients entered on the protocol will receive 5 mCi of Indium 111-MX-DTPA huBrE-3(2 mg) plus 48 mg of nonconjugated BrE-3 intravenously over one hour. Images and pharmacokinetics will be performed over the course of one week. In addition, conventional clinical evaluation for toxicity will be performed at baseline, during infusion and at 72 hours after infusion consisting of history, physical examination, and routine blood tests. Anti-antibody response will be assessed at baseline, day 8, 5 weeks, and 3-6 months post antibody administration.

It is likely that we will be able to begin to treat patients by 9/95. We are currently in the process of screening 16 potentially eligible patients for BrE-3 Ag by retrieving tissue from there original surgical specimen and measuring serum levels of BrE-3. We anticipate that we will be able to complete this protocol within twelve months. Following this study, we plan to initiate a radioimmunotherapy trial with ^{90}Yttrium-huBrE-3 alone, or in combination with potentially synergistic agents such as topo or with IL-1 as described in the original grant proposal.

We have done preliminary work to determine the distribution and toxicity of BrE-3 ^{90}Y conjugate. Human breast tumor xenografts were grown in Balb/c nude female mice. Mice were treated with between 150 μCi- 355 μCi ^{90}Y MX-DTPA BrE-3. From these preliminary studies we were able to define the maximum tolerated dose of ^{90}Y MX-DTPA BrE-3 as 266 μCi ^{90}Y in this murine system and expect that 200 μCi will be the starting dose for the combination studies in animals. The potential enhancement of therapeutic index of radioimmunotherapy with a breast-tumor directed radioimmunoconjugate by the addition of a topo-1 inhibitor such as topo or 9-aminocamptothecin will be examined in an animal model of human breast cancer. This xenograft model will help determine the optimal schedules of administration of each modality, and permit preliminary estimates of effects on toxicity. It is expected that this experimental work will provide the basis for a clinical Phase I trial in patients with advanced breast cancer which will capitalize on the advantages of the more effective topo-1 inhibitor chosen, the targeting capability of a radioimmunoconjugate, specifically ^{90}Y- MX-DTPA huBrE-3, and the anticipated synergism between the two therapies.

Preliminary to the combined modality studies, over the last year we have completed several experiments in the mouse model in an attempt to evaluate the efficacy of continuous infusion topotecan. In the fall of 1994, we performed the first experiments on N/PLOR male mice six to eight weeks old. We used 6 mice per

experimental group. On day 0, each mouse was injected with 3×10^5 of syngeneic mammary tumor MGT cells subcutaneously. On day 1, two groups of 6 mice each had water/topo pumps (Alzet pumps) implanted under the skin, through a small incision. Each pump was programmed for infusion of up to seven days. After the placement of the pump, the skin was stapled and the wound was disinfected with betadine. One group of mice received saline alone and one group received topo at 2.0 mg/m^2 continuous infusion over seven days. One group of mice served as controls and did not receive pumps. At each timepoint for up to 20 days, the topo group had lower tumor volumes than both the saline pump group and the control mice. This is shown in figure 1 in the appendix. Over the next several months these experiments were repeated for verification. A later experiment performed in January, 1995 failed most likely because the Avertin was not fresh and was subsequently reprepared. In addition, the topo dose was higher at 2.4 mg/m^2.

We then performed experiments to evaluate the effect of a more prolonged infusion topotecan on tumorigenesis. On day 0, all mice received 3×10^5 MGT cells injected subcutaneously. On day 1, two groups of six mice each had implantation of the Alzet pump as described above. One group received a saline infusion and the other group received a topo dose of 2.0 mg/m^2. Another group of six mice served as controls (did not have pump implantation). On day 10, a second Alzet pump was inserted in the first two groups of mice with, one group receiving saline and the topo group continuing to receive topo at 2.0 mg/m2 over seven additional days. Because the pumps were not available on day 8, the topo group had a two day period of no drug. The results of this experiment is shown in figure 2 in the appendix. At each timepoint tumor growth in the topo group was inhibited.

The same experiment was repeated in the Spring of 1995, with the exception that male mice were used, and the second pumps were inserted on day 8. In this experiment, the topo group of mice had a continuous infusion of topotecan over 14 days. The results of this experiment is shown in figure 3 in the appendix. In this experiment there was overlap of the tumor volume in the control group without pumps and the topo group. The reason for this was not clear.

The next set of experiments have incorporated IL-1 to assess whether IL-1 has any synergistic effects on tumor growth inhibition with topo and to assess toxicity. Female mice, 6 to 8 weeks old were used in five groups of six randomly selected mice each. On day 0 each mouse was injected with 3×10^5 MGT cells s.c.. On day 1, two groups of mice received IL-1, 0.25 ml intraperitoneal (0.5 ug/mouse). IL-1 alpha rhu has been supplied from Immunex. It is packaged as 100 ug suspended in 0.2 ml saline-BSA. Each vial contains 100 ug-5×10^8 IL-1, 40 mg mannitol, 10 mg sucrose and 1.2 mg tris. Four hours after the IL-1 injection, Alzet pumps were inserted. Two other groups of mice also had Alzet pumps inserted on day 1. As. above the topo dose used was 2.0 mg/m^2. Group 1 received saline pump alone. Group 2 received IL-1 followed by the saline pump. Group 3 received IL-1 followed by topo pump infusion. Group 4 received topo pump infusion alone. Group 5 served as control and did not have pump insertion

or IL-1 injection. On day 8, new pumps were implanted in the topo animals to receive another 7 day infusion of 2.0 mg/m^2. As shown in figure 4 in the appendix, there was no obvious difference in tumorigenesis among the various groups.

Subsequently, we have performed an experiment using IL-1 in a continuous infusion. Other experiments carried out by our colleagues had demonstrated that continuous infusion of IL-1 yielded a higher therapeutic index, lower toxicity and greater hematologic reconstitution than bolus injection. Male mice, 8 to 10 weeks were used. On day 0, five groups of six mice each were injected with 3 x 10^5 MBT cells, s.c. On day one, four groups of mice had pumps implanted as described above. Each pump was prepared as follows:

	EMPTY	TOPO	IL-1	TOPO + IL-1
Topo	-------	35 ul	-----	35 ul
Saline-BSA	65 ul	65 ul	15 ul	15 ul
IL-1	--------	-------	50 ul	50 ul
Water	35 ul	-------	35 ul	-------

The effect of the infusions on tumorigenesis is shown in figure 5 in the appendix. Although not dramatic, the topo plus IL-1 group had the most tumor growth inhibition. White blood cell counts were performed on the IL-1 and IL-1 plus topo group at baseline and on day 5. The WBC count increased by approximately 2-3 fold.

In the coming funding period, we intend to begin combination studies with topo and ^{90}Y MX-DTPA BrE-3 in the nude mouse xenograft model, (human breast cancer cell lines ZR-75 or MX-1). The initial combination studies will examine the possible toxicities that could be additive. We will start at a dose slightly below the MTD(200 uCi BrE-3) defined in our preliminary experiments with this xenograft model. Dosing levels of topo will be selected on the basis of measurement of adequate topo levels by the Alzet pump in the current experiments. Mice will be followed for up to 4 weeks with the BrE-3 dosing regimen set at an initial dose one week after the topo administration. If substantial toxicities result, we will test a dose fractionation schema once a week with half or one third the dose of BrE-3. Based on the acceptable toxicity profile of combination topo and radioimmunoconjugate, the timing sequence of these modalities will be examined with regard to efficacy on tumor growth inhibition. Each of the experiments will incorporate 4 arms: control mice with no drug administration, topo alone, ^{90}Y-MX-DTPA BrE-3 alone and ^{90}Y-MX-DTPA BrE-3 plus topo. Again, animals will be followed for up to 4 weeks. Steady state drug levels of topo and tumor drug levels will be followed. In addition, tumor distribution of BrE-3 will be assessed. Tumor growth will be measured in all the groups and efficacy will be determined by reduction in tumor size. If the IL-1 decreases toxicity and/or improves efficacy, it will

subsequently be incorporated into the preclinical studies. Ultimately, we plan to use the data generated in these combination studies to develop a clinical trial.

Conclusion

In summary, over the last year we have made progress in obtaining a humanized BrE-3 monoclonal antibody chelated to ^{111}Indium which is ready to begin phase I study testing. We plan to initiate a radiotherapeutic trial with ^{90}Y huBrE-3 as soon as we obtain preliminary data regarding safety and feasibility on the ^{111}Indium study. In the original grant application, we planned to initiate a radiotherapeutic study with the murine antibody because we did not expect to have the humanized antibody available. For the reasons outlined above, the humanized antibody has greater potential for therapeutic efficacy and we look forward to begin these studies within the next month.

We plan to initiate combination studies with targeted radioimmunotherapy and chemotherapy, specifically the topo I inhibiter, topotecan. Preliminary work in the mouse model performed this year demonstrated that prolonged infusion topotecan can be administered safely to mice and has antitumor activity. The addition of IL-1 had variable results described above. Over the upcoming year we plan to do more extensive studies in nude mice bearing human tumor xenografts with combination radioimmunotherapy and chemotherapy. We will continue to explore the potential role of IL-1 as a radioprotector. Ultimately, we hope to conduct a clinical trial using combination topotecan and ^{90}Y-huBrE-3.

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Appendixes

1- Protocol:

A phase I study of the toxicity , imaging and dosimetry of a humanized breast-directed radiolabeled monoclonal antibody (BrE-3).

2- Figures:

1-Effect of topotecan pumps on MGT
2-Effect of topotecan on MGT tumorigenesis
3-Effect of topotecan on MGT tumorigenesis
4-Effect of topo and IL-1 on MGT tumorigenesis
5-Effect of topo and IL-1 on MGT tumorigenesis

A PHASE 1 STUDY 0F THE TOXICITY, IMAGING AND DOSIMETRY 0F A HUMANIZED BREAST-DIRECTED RADIOLABELED MONOCLONAL ANTIBODY (BrE-3)

NYU Medical Center

Co-Principal Investigators:	Carolyn Wasserheit, M.D. Elissa L. Kramer, M.D.
Associate Investigators:	Howard Mizrachi, M.D. Jessie Sullivan, M.D. L. Liebes, Ph.D. Marilyn E. Noz, Ph,D. Susan Zolla-Pazner, Ph.D. Phil Furmanski, Ph.D.
Biostatistics:	Ann Zeleniuch-Jacquotte, M.D., M.S.
Consultants:	Roberto Ceriani, Ph.D., M.D. Jerry Peterson, Ph.D.

1.0 OBJECTIVES

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 top determine the nature of this response

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

2.0 INTRODUCTION AND BACKGROUND

Breast cancer is the most common malignancy in females in this country and accounts for approximately 18% of all cancers in women. It is also the second most common cause of death from cancer in women with an estimated 43,000 deaths per year. Although survival figures appear to have improved for patients with localized disease at presentation with the advent of adjuvant chemotherapy, the long-term outlook for patients with advanced, recurrent, or metastatic disease remains poor. Indeed, no curative treatment regimens have been developed for these type of patients.

With this in mind, there is an obvious need for newer modalities of treatment to be used separately from or in combination with current modalities. One such potential modality is that of radiolabeled monoclonal antibodies. Although monoclonal antibodies directed against tumor-associated antigens have been used as therapeutic agents in a variety of malignancies (1 -13), this approach has yet to be exploited in the treatment of breast cancer.

Several monoclonal antibodies against human breast cancer antigens have been produced. Antibodies which react strongly to human breast cancers and recognize antigens referred to as human milk fat globule (HMFG) antigens or breast epithelial mucin include the BrE-3, MC-5 and KC4G, and Mc3 antibodies (15, 16). Investigations have shown three of these antibodies (KC4G, BrE.3 and Mc5) recognize similar high molecular weight (>400,000) antigens present on many breast cancers. Mc3 recognizes a 46 kD epitope of breast epithelial mucin. These murine antibodies can be effectively radiolabeled and thus are candidates to be used in the treatment of human breast tumors. Three of these antibodies have already undergone Phase I testing in patients with breast cancer. The experience generated in clinical studies with MC5, KC4G and now murine brE-3 has provided important information for designing studies with BrE-3. This protocol provides for the Phase I testing of the humanized BrE-3 antibody which has been radiolabeled with ^{111}Indium. These studies will determine how well the antibody localizes to known sites of disease and will evaluate its toxicity, pharmacokinetics, and therapeutic effect. It is expected that humanized BrE-3 will have similar targeting properties to the murine BrE-3 since the affinity is similar although the area under the curve for serum may change for the humanized version and therefore, alter the amount of antibody available for tumor localization. Also,tumor to nontumor ratios may differ as may organ biodistribution because of altered physiologic handling of the human IgG isotype.

2.1 .5 Prior clinical studies with antibodies similar to BrE-3.

The University of Colorado performed a dose escalation study with unlabeled KC4G antibody. They gave 10-500 mg once weekly for 4 weeks in non-small cell lung cancer patients. In one study in 15 patients treated there was one minor response with no partial or complete tumor responses.

Toxicity from monoclonal antibody therapy has overall been mild. They have observed occasional low fever and mild allergic reactions. In their recent experience, of 45 patients treated thus far, three have had reactions attributed directly to KC4G infusion with no long term sequelae. One patient had transient hypotension immediately after beginning his fifth infusion of antibody. He had received a total dose of 230 mg antibody and developed high titers of human anti-mouse lgE and IgG. The second patient developed an acute dyspnea reaction 2 1 /2 hours into a 5 hour infusion. His total dose received was 780 mg and he developed high titers of human anti-mouse lgG but not lgE. The third patient developed a clinical syndrome compatible with serum sickness after 2,000 mg of total KC4G. She developed low titers of human anti-mouse IgG but no IgE. Other patients developed low titers of human anti-mouse lgG, but experienced no clinical symptoms.

2.2.1 Prior studies with 131I radiolabeled MC5 monoclonal antibody.

Dr. Ceriani and his coworkers demonstrated that ^{131}I-Mc5 was useful for imaging and therapy of human breast cancers in heterotransplanted into athymic nude mice. Based on their preclinical data the Univ of Colorado initiated a clinical imaging trial of radiolabeled Mc5 in patients with advanced breast cancer. The University of Colorado has studied 12 patients with metastatic breast cancer with 40 - 400 mg of MC5 labeled with 5 millicuries of ^{131} I. Toxicity has been minimal and consists mainly of mild allergic reactions such as hives, pruritus, low grade fever and arthralgias. All have been reversible. To date they have been able to obtain images of known tumor sites in only one patient who received 400 mg of radiolabeled Mc5. They have shown that high levels of circulating Mc5 antigen were present in these patients. They believe that failure to image other patients may be due to high levels of circulating antigen which prevent the antibody from reaching the tumor. In view of this, values of the circulating antigen identified by BrE-3 were obtained in the same patients participating in the Mc5 protocol using a radioimmunoassay, and it was found that except in one patient, the circulating BrE-3 levels were only 5-15% of those for Mc5. Limited radioimmunolocalizaton studies with either radioiodinated or ^{111}In were performed at the University of Colorado

2.2 Preclinical Studies of Unlabeled and Radiolabeled Monoclonal Antibody BrE-3.

The monoclonal antibody to be used in this study was created by a fusion of HMFG stimulated spleen lymphocytes of the NZB mouse strain and 653 mouse myeloma. The MoAb is an IgG1, arising from a hybridoma with a high level of BrE-3 MoAb production in serum-free medium.

Binding of BrE-3 to breast carcinoma cell lines, grown in vitro and in vivo.

Twenty human breast carcinoma cell lines maintained for many years in in vitro culture were positive for BrE-3 staining in terms of prevalence (% of positive cells) or intensity of staining. When several of these lines were also grown as tumors in immunodeficient mice they also expressed high levels of the BrE-3 antigen. Prevalence of BrE-3 binding of human breast tumors detected by flow cytometry reveals close to 100% positive cells.The intensity of staining with BrE-3 varied widely for the different cell lines; however, most cell lines had relative channel numbers above 500 (the range was 30 to 3300). In some cases, the intensity could only be attributed to membranous staining, others to cytoplasmic; however, in most cases it was a combination of both. Other carcinoma cells known to express the BrE-3 Ag were also positive (SKOV-3, ovary; PANMIA, pancreas: HT-29, colon).

In summary, all breast tumor cell lines tested bound BrE-3 and at a very high level, both when they were cultured as monolayers or carried in vivo as tumors in immunodeficient mice.

Normal and neoplastic human tissue distribution of BrE-3 by histopathology.

Studies were carried out in formaldehyde fixed, paraffin-embedded tissue blocks. Binding of BrE-3 to normal human tissues, was restricted. BrE-3 bound weakly (as compared to breast tumors) to the alveolar lining of lung, some kidney tubules, pancreas and stomach. Normal human breast tissue, was negative. In contrast, many human epithelial tumors were positive for BrE-3. Most, if not all, breast tumors were positive as well as tumors of lung and ovary.

Radioimmunotherapy (RIT) with BrE-3 conjugates in mice

After a dose of 500 uCi, (considered maximal tolerated dose) of 131I-BrE-3 was given to mice carrying MX-1 tumors (approx. 100 mm3), tumor volume was not regained for close to a month, while from there on the tumor growth was resumed sluggishly. The % inhibition of growth (% IG) was 90.2%) at 28 days. No deaths were recorded in the experimental group.

Using a ^{90}Y-conjugate of BrE-3 (Coulter) the results of RIT were even more surprising. Tumor destruction was very large after 1 week and the tumors (4 out of 4) seemed to be eradicated after 20 days (Graph 11). The ^{90}Y-BrE-3 was injected when the tumors were 100mm^3 and the control, untreated tumors are as an average 1162mm^3 in volume at 40 days. This curative effect of BrE-3 was obtained with 250 uCi of the ^{90}Y-BrE-3 conjugate. No deaths were recorded in the experimental group.

Based on these preclinical studies investigation of the BrE-3 antibody in humans has begun.This initial study will radiolabel the antibody with a relatively low dose of radioactivity and will determine localization, pharmacokinetics, and radiation dosimetry, as well as the toxicity of antibody therapy. Subsequent studies with this antibody will then utilize higher doses of bound radioactivity.

In all the studies cited above, both unmodified MoAb administration and infusion of isotope labeled MoAb for imaging purposes has been associated with minimal or no toxicity. In the cases treated with anti-idiotype antibodies, toxicities generally occurred in patients who had circulating tumor cells, free serum idiotype protein levels of >1 ug/ml, or an anti-mouse antibody response (3). Patients who did not have target protein or cells in the blood at the time of infusion could tolerate antibody infused at rates as high as 200 mg/h without side effects. The principle side effects experienced included fever, chills, dyspnea, headache, nausea, vomiting, diarrhea, and myalgias. All of these effects were fully reversible and often responded to the slowing or discontinuation of the antibody infusion. Up to 900 mg of antibody could be given with relative safety. Other toxicities which were even less common were mild hypotension, persistent facial palsy,mild and reversibly azotemia, thrombocytopenia, neutropenia, and transaminase elevation. We have detailed adverse reactions experienced with antibody KC4G including our evaluations of human anti-mouse IgG and lgE development .

2.3 Phase I radioimmunolocalization trial with ^{111}In MX-DTPA BrE-3

We have recently completed a Phase I localization trial with ^{111}In - MX-DTPA BrE-3, a murine monoclonal antibody of the IgG1 isotype, directed against the 400 kD epitope of breast epithelial mucin. This antibody has been shown to bind to >90% of human breast carcinomas against which it has been tested. BrE-3 antibody is one of a family of antibodies developed by Dr. Roberto Ceriani at the Cancer Research Fund of Contra Costa and is available from Coulter Immunology in both the nonconjugated form and as the Methyl benzyl isothiocyanate DTPA conjugate for radiometal labeling. This Phase I localization trial in patients with advanced metastatic breast carcinoma was performed at NYU and in cooperation with Dr. Sally DeNardo at U.C. Davis. In the course of this trial we described the pharmacokinetics, biodistribution, radiation dosimetry, and potential for

tumor localization of ^{111}In - MX-DTPA BrE-3. There were 70 known sites of tumor. An additional 11 sites of abnormal radioactivity accumulation were seen of which 5 could be confirmed as metastatic disease. Overall 87% of the known lesions were detected by antibody imaging. 91% of the known skeletal metastases were detected. Biexponential modeling of radiolabeled antibody in serum showed a $T1/2\alpha = 9.5 \pm 2.7$ hrs. and $T1/2\beta = 56 \pm 25.4$ hrs. Total urinary excretion averaged 35.5 ±19.3 % injected dose (ID) by day 8. Quantitative imaging showed that 0.02-2.56 % ID localized in tumors. Preliminary tumor radiation dosimetry estimates extrapolated from the ^{111}In-BrE-3 to ^{90}Y-BrE-3 ranged widely but averaged 147 rads/mCi administered. Grade I-II allergic reactions were encountered in 3 of 15 patients and HAMA was detected in 7.

Based on these results, a Phase I therapy trial with ^{90}Y-MX-DTPA-BrE-3 has been started in previously treated patients with advanced metastatic or recurrent breast cancer. To date, the initial dose level (6.25 mCi/m2) has been completed with Grade I thrombocytopenia observed in one patient. A minimal response was observed in one patient. Increments of 3 mCi ^{90}Y/m^2 are planned. We anticipate that MTD will be reached at the third or possibly fourth dose level and that the dose limiting toxicity will be hematologic. In the course of this trial, we are coinfusing ^{111}In-MX-DTPA-BrE-3 at a constant dose. Serial imaging and blood and urine collection are performed to measure biodistribution and pharmacokinetics. We are monitoring marrow hematopoietic progenitors in the marrow before and after radioimmunoconjugate therapy to determine the marrow response to the radioimmunoconjugate. Bone marrow aspirates and biopsies are analyzed also for ^{111}In and ^{90}Y content to determine the relationship of the biodistributions of the two radioisotopes.

Rationale:

Two immediate obstacles arise in the delivery of effective radioimmunotherapy of breast cancer. One is the hematologic dose limiting toxicity; the other is the immunogenicity of the antibody. One approach to diminishing toxicity has been to employ a dose fracitonation scheme with weekly radioimmunoconjugate administrations over a period of two to three weeks. However, with an immunogenic murine antibody, the pharmacokinetics and targting of tumor may be altered by the presence of an anti-antibody response. Furthermore, in patients who do respond to radioimmunotherapy, a repeat cycle may be prevented by the presence of anti-antibody formation. Thus, we propose to use a humanized BrE-3 antibody in which both the constant and 80% of the variable portion of the antibody is humanized and only the CDR's remain murine. Humanized BrE-3 was developed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani. This antibody has been shown to have three times the affinity of the murine BrE-3. Based on the favorable characteristics of the murine BrE-3, we believe that exploration of the localization characteristics, pharmacokinetics, radiation dosimetry, and immunogenicity of the ^{111}In-MX-DTPA humanized BrE-3 will provide information concerning the radioimmunoconjugate's potential as a therapeutic agent in breast cancer in a dose fractionation schema or in repeated cycles of radioimmunotherapy.

3.0 Overview

The objectives of this study are to determine the extent of antibody localization in metastatic breast cancer, to develop radiation dosimetry estimates for Indium-111 labeled humanized MoAb, to assess toxicity of the radiolabeled MoAb and to assess the immunogenicity of the radiolabeled humanized antibody. 15 patients with advanced breast carcinoma will receive Indium-111 labeled MoAb. Images and pharmacokinetics will be performed over the

course of a week. When there is readily accessible disease, tumor biopsy will be obtained to assess tumor localization of antibody. Follow-up serum samples will be obtained to measure human anti-antibody response. For this protocol we will use ^{111}In MX-DTPA BrE-3. The antibody has already been humanized at the Cancer Research Fund of Contra Costa and will be provided in form acceptable for human use by Coulter Immunology.

Schema

Number of	Dose BrE-3(mg)		Radioactivity.	
Patients	Unlabeled	Radiolabeled *	Isotope	Dose(mCi)
15	40	10	^{111}In	5

* 2 mg of huBrE-3-benzyl methyl DTPA-^{111}In Chelate and 8 mg of nonconjugated unlabeled huBre-3.

4.0 PATIENT ELIGIBILITY CRITERIA

1. Patients must have a histologically confirmed diagnosis of carcinoma of the breast.
2. Patients must have recurrent or metastatic disease.
3. Brain involvement will not exclude patients from study, but must have been treated or under treatment with standard radiation therapy.
4. Patients must have breast cancer which is reactive with the treatment antibody. At least 25% of tumor cells within a biopsy specimen must be reactive with the antibody.
5. Patients must have evaluable and/or measurable disease, exclusive of brain disease.
6. Patients must be fully ambulatory with a life expectancy of greater than 3 months and a > 60 Karnofsky performance status.
7. Patients must have normal renal function (creatinine < 2.O mg/dl) hepatic function (bilirubin <3.0 mg/dl).
8. Patients must be without evidence of active collagen vascular disease, vasculitis, rheumatoid arthritis, glomerulonephritis, New York Heart Association class 3 or 4 heart disease (severe heart disease) and/or other serious illnesses.
9. Patients must not have received prior therapy with murine monoclonal antibodies.
10. Patients must be off cytotoxic therapy for 10 days prior to administration of antibody and for one week following.
11. Patients must have the ability to understand and sign an informed Consent Form.
12. Eligible women of child bearing potential must have used adequate contraception or have a negative pregnancy test.
13. Patients must be > 18 years of age.

5.0 DRUG INFORMATION

The monoclonal antibody will be provided by Coulter Immunology, Hialeah, Florida, in a final concentration of about 40 mg/ml under contractual agreement with NCI. Purified product will be produced in serum free media.Protein A purification will be performed and the antibody will be sterilized through a 0.2 u filter. General safety, sterility, pyrogenicity, polynucleotides, mycoplasma, and adventitious virus contamination will be tested in accordance with a Notice of Claimed lnvestigational Exemption for a New Drug (IND) (Office of Biologics, U.S. Food and Drug Administration). All clinical studies will be performed with material prepared under an IND.

The methyl benzyl DTPA conjugated BrE-3 for radiolabeling will be provided by Coulter Immunology, Hialeah, Florida in a concentration of 2 mg/ml. Thje radiolabeling kits supplied by Coulter include sterile, low pyrogenic solutions of acetate buffer and calcium EDTA in saline. The Indium-111(sterile, low pyrogenic) will be purchased INS.IPA from Mediphysics, Inc.

5.1 Immunoconjugation

Radiolabeling of antibody will be performed under the supervision Dr. Elissa Kramer in the laboratory located in the Division of Nuclear Medicine of Tisch Hospital. A Methyl benzyl DTPA huBrE-3 conjugate will be provided by Coulter immunology. Methyl benzyl DTPA BrE-3 will be radiolabeled under an IND protocol by mixing 2 mg of the antibody chelate with 5-10 mCi of acetate buffered $^{111}In\ Cl_3$ for 20 minutes at room temperature.

A 0.1 ml of the product will be withdrawn for radiochemical purity and immunoreactivity determinations. The remaining will be diluted to 18 ml with USP injectable grade O.9% saline solution containing 5% human serum albumin. Three milliliters of this final product will be withdrawn for USP sterility testing and for LAL testing.

The radiochemical purity of the labeled antibody will be determined by ITLC chromatography using O.9% saline eluant. The radiochemical purity of the final product will be >90% for patient administration. The above two criteria will be met before the labeled antibody can be administered to the patient. Immunoreactivity of this preparation will be determined using an antigen bead radioimmunogen procedure by applying infinite antigen excess (17). The minimum acceptable immunoreactivity will be 50%. The LAL test for pyrogens will be negative for patient dosing. The sterility test will be performed according to the guidelines prescribed in the United States Pharmacopeia.

5.2 Storage

The radiolabeled MoAb huBrE-3 will be stored at 2° - 8° C in a shielded environment while the quality control procedures are in progress. Upon receiving a satisfactory report about the quality of the labeled MoAb, it will be made available in the Department of Nuclear medicine for administration to the patient.

6.0 STUDY DESIGN

All patients will undergo an evaluation for the purposes of determining and measuring other sites of evaluable disease. This includes a thorough history and physical examination, blood counts and chemistry surveys, routine chest x-ray and electrocardiogram, and urinalysis, computerized tomograms (CT scans) of appropriate areas, and bone scan. In addition, assays for free circulating antigen, recognized by either BrE-3 and Mc3, will be performed, as well as assays for human antibodies which react with these antibodies. The assay for free antigen will be performed by Dr. Roberto Ceriani, Cancer Research Fund of Contra Costa, Walnut Creek, CA.

Some of the initial baseline observations will be repeated during the course of the study to assess toxicity, response to therapy, and immunologic alternations. This includes CBC, chemistry, urinalysis, and anti-antibody.

6.1 Antibody administration

Patients accepted on this protocol will receive a single "imaging dose" of labeled antibody to establish biodistribution patterns of this antibody, the degree of immunodetection, and dosimetric parameters,as well as to detect any unforeseen, exclusionary toxicities.

6.1.1 For this Phase I study all patients will be out-patients at Tisch Hospital. They will be monitored in the Division of Nuclear Medicine on the second floor of the hospital up to 6 hours post infusion. All patients will receive 5 mCi of ^{111}In hu BrE-3-methyl benzyl DTPA as detailed in the schema followed by serial gamma camera images during the first week through day 8. After the imaging dose is given, dosimetric evaluation studies will also be performed on each patient. Each patient will receive a total of 50 mg of antibody.

Patient preparation

6.1.2 Hypersensitivity skin testing will not be performed prior to each antibody infusion in each patient. Data from the National Cancer Institute suggests no correlation between local erythema and induration at the site of skin tests and subsequent systemic

allergic reactions to mouse antibody infusions (K.Foon, personal communication). Skin testing may also increase the likelihood of sensitization to mouse antigens.

Infusion

6.1.3 Intravenous tubing will be pretreated with 0.9% NaCI and 5% human serum albumin. After placement of an intravenous line, BrE-3, at the dose described above,will be infused as follows:

Total mg	mg Ab unlabeled	volume infused unlabeled (ml)	mg Ab labeled	volume infused labeled (ml)	Total volume infused (ml)
50	40	235	10	15	250

Unlabeled antibody will be infused initially. Thirty minutes after completion of the unlabeled antibody infusion, the ^{111}In-labeled antibody will be infused. The infusion will have a total volume of 250 ml of O.9% NaCI and 5% human serum albumin and will be infused over 2 hours depending on rate-dependent side effects. Vital signs will be taken every 15 minutes during the infusion, and every one hour post infusion until stable. A thorough cardiopulmonary physical examination will be done prior to and at the conclusion of antibody infusion. Medications including acetaminophen, diphenhydramine, epinephrine, and corticosteroids will be kept at hand for treatment of allergic reactions should they occur.

7.0 Treatment Plan Modification

7.1 Toxicities which may be conceivable following the intravenous administration of murine-derived monoclonal antibody include all of the well-recognized allergic reactions to foreign protein, including fever, urticaria, bronchospasm, anaphylaxis, Arthus reaction, vasculitis and serum sickness. Additional possible toxicities include renal and hepatic dysfunction, immune cytopenia and coagulopathy. Furthermore, the administration of large quantities of aggregated lgG could result in anaphylactoid reactions including chest and back pain, hypotension and bronchospasm.

7.2 Toxicity Grading: All toxicities will be graded according to the attached scale (See Appendix)

7.3 Off Study criteria

1. Unacceptable toxicity defined as grade 3 or 4. With grade 3 or 4 toxicity, the infusion will be stopped and no further antibody administered.
2. At the patient's or investigator's discretion.
3. Patients with grade 1 or 2 toxicity may continue on study at the discretion of the investigator.

7.4 Previously Unrecognized Toxicity. To be reported to Principal Investigator, FDA and NCI.

8.0 STUDY PARAMETERS AND SERIAL OBSERVATIONS

8.1 Initial Evaluation

The initial patient evaluation will include:

8.1.1 Complete history and physical examination including height, weight and performance status.

8.1.2 Complete CBC including differential blood count and platelet count, PT, PTT.

8.1.3 Electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT.

8.1.4 Urinalysis

8.1.5 Chest x-ray, and other x-rays or scans as clinically indicated to document state of disease.

8.1.6 EKG

8.1.7 A serum sample (10-15 cc) will be collected pre-injection, aliquoted, and frozen at -20° for determination of human anti- antibody and free antigen. (Other samples will be collected at other times points for storage as shown in the serial observations).

8.1.8 Immunohistochemical testing of tumor biopsy with quantitative evaluation of % cells positive for antibody staining and intensity (1-4+).

8.2 Serial Observations and Laboratory Monitoring Schedule

8.2.1 Blood samples will be obtained for analysis of radioactivity and for RIA analysis of BrE-3 clearance immediately, 5, 60, 120 minutes, , 6, 24, 48,and 72, 96, 144, 168 hours post injection. Blood for Human anti-antibody will be obtained at Day 8,5 weeks, and 3-6 months post infusion.

8.2.2 Urine samples will be collected from O-2, 2-6, 6-24, 24-48, 48-72, 72-96,96-120, 120-144 and 144-168 hours postinfusion.

8.2.3. Complete CBC including differential blood count and platelet count, PT, PTT, electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT, and urinalysis will be obtained at 72 hours after antibody administration. Values which become abnormal at this time point in comparison to baseline values will be repeated to assess normalization.

8.2.4 Blood and urine samples will be collected by the nursing staff and sent to the appropriate laboratories.

8.3 Imaging

The regional gamma camera images will be obtained at 2, 24, 48 and/or 72 hours and 8 days using a large field of view gamma camera. Data for each image will be acquired by gamma camera over a 5-minute period (7.5 minutes for Day 8 images) and transfered to optical disk linked to a SUN computer for later analysis. The data will be processed and stored with a dedicated computer which is available in the Nuclear Medicine Department to measure regional uptake of radiolabeled BrE-3 in major organs, tumor, and blood pool at the times designated above. Analysis of images will include counts over critical regions of interest comparing tumor: normal tissue ratios to aid in assessing the feasibility of using this agent for tumor detection.

In addition SPECT imaging will be performed at 72 hours and/or 1 week post administration.

8.4 Measurements for dosimetry estimations

8.4.1 Serum specimens will be obtained for analysis of radioactivity (as well as for RIA analysis of BrE-3 clearance) immediately, 5, 30, 60, 120 minutes, 4, 6, 24, 48,and 72 hours post infusion and then daily X 4. The radiochemical form of radioactivity in plasma samples obtained at 120 minutes and 24 hours will be analyzed by HPLC. Urine will also be collected daily for seven days in containers labeled as radioactive and delivered to the Division of Nuclear Medicine for renal clearance analysis of radiolabeled antibody.

8.4.2 Serial quantitative imaging as described in section 8.3 will be performed. Transmission scans using an ^{111}Indium filled flood source are performed prior to antibody administration for attenuation correction. Conjugate anterior and posterior regional gamma camera images are performed at each time point. In our department, ^{57}Co fiducial markers are used to improve the reproducibility of the positioning between imaging sessions. An additional energy window centered on the 122 keV window to provide simultaneous images which demonstrate the position of the markers for later digital alignment of serial images. A standard containing a known amount of ^{111}Indium is also imaged at each imaging session to provide information on sensitivity and for calibration of the gamma camera data.

8.4.3 To aid in estimating tumor accumulation of radioactivity, readily accessible tumor may be biopsied on Day 8. These biopsies will be performed by Dr. Matthew Harris or Dr. Daniel Roses.

9.0 STATISTICAL CONSIDERATIONS

This imaging study is designed as an evaluation of the tumor localization potential, radioimmunotherapeutic potential, and immunogenicity of the humanized antibody. The optimal scanning conditions will be determined by the Nuclear Medicine Department after consideration of maximal tumor uptake versus nonspecific background uptake. Statistical comparisons with conventional methods of tumor staging will be performed in subsequent study. Images will be reviewed for technical quality and for localization of the imaging agent at the sites of known tumor.
The Pharmacokinetic data will require statistical support for modeling of antibody distribution and radioisotope clearance in radiolabeled studies. Special purpose programs are available and will be modified as necessary in order to make dosimetry estimates.

10.0 METHODS

11.2 Image Analysis/Pharmacokinetics/Dosimetry Estimates

10.2.1 Pharmacokinetic Analysis
The pharmacokinetic analysis of labeled and unlabeled monoclonal antibody movement into the tumor and through each patient will provide a manageable and useful summary of the data that are collected. The pharmacokinetic analyses will:

1) Increase the pharmacological and physiological understanding of the usefulness of the monoclonal antibody as an imaging agent.

2) Provide information about potential for this antibody to target therapeutic doses of radioactivity to tumor.

10.2.2 General Approach
The efficiency of elimination of the monoclonal antibody via the urine, and in toto from the blood, will be described using renal antibody clearance and total antibody clearance from the blood, respectively. Clearance values reflect the volume of blood from which the monoclonal antibody is removed per unit time (hence, ml/min) by the organ(s) of interest. Based on this information, gathered and interpreted for labeled monoclonal antibody, radiation doses and dose interval of monoclonal antibody will be calculated using standard mathematical theory describing multiple (repeated) dosing. The above-mentioned pharmacokinetic approach is quite standard and is available in standard text (43).

10.2.3 Biodistribution
In order to study the distribution of conjugated monoclonal antibody, multiple regional scintiphotos will be obtained at 2, 24, 48, and 72 hours and one week after injection in the Nuclear Medicine Department. Regions of interest will be established and counts per pixel calculated for those organs which have significant uptake and for a background region in order to calculate relative uptake ratios. Previous studies have demonstrated significant accumulations of radioactivity in liver, spleen, kidneys, and lungs. In addition, serial blood sampling will provide an estimate of whole body distribution of radiolabel.

Attenuation of activity will be corrected for by transmission images obtained just prior to antibody administration for each patient using a flat field source filled with a known concentration of ^{111}In. Alignment of digital transmission and emission images will be

performed using fiduciary markers and a two-dimensional image registration algorithm within *qsh*, an image handling toolkit which runs under a UNIX operating system. Activity within an organ will be determined using the geometric mean obtained from conjugate views for larger organs and a single planar view for smaller organs (e.g. kidney and spleen) and tumors and then corrected for attenuation and background. The consistency of detection sensitivity of the camera will be checked on a daily basis at the time of the patient imaging by imaging a standard at a fixed distance to yield a system calibration factor (32a). From this data, the fraction of the radiolabel resident in each organ can be closely estimated.

10.2.4 Counting of Urine/Serum Biopsies/Pharmacokinetics

In general, the goal of these studies is to provide better understanding of the kinetics of tumor localization. For each infusion of radiolabeled monoclonal antibody, blood samples will be drawn at 5 minutes, 1 , 2, , 6, 24, 48, and 72, 96, 144 and 168 hours postinfusion. Urine samples will be collected from O-2, 2-6, 6-24, 24-48, 48-72, 72-96,96-120, 120-144 and 144-168 hours postinfusion.

Pharmacokinetic analysis will be performed by Dr. Liebes in the Oncopharmacology Laboratory. The distribution of monoclonal antibody will be followed by radioactivity determinations from the timed blood samplings. Total radioactivity (dpm/ml) will be measured in each blood sample and urine sample. We will count aliquots of blood and urine obtained at multiple time points in comparison to a standard made from the administered dose. This is then expressed as % injected radioactivity cleared or excreted over time, allowing for normal radioactive decay by standard decay formulas. Serum samples are subjected to gel permeation HPLC to determine the percent radioactivity associated with immunoglobulin, associated with high molecular weight complexes (presumably antigen-antibody complex), and small molecular weight moieties.

These will be converted into monoclonal antibody concentration based on the specific activity determination of the labeled BrE-3 antibody . Nonlinear pharmacokinetic models which run on a PC , PCNONLIN (Statistical Consultants, Lexington,Ky) will be used for the analysis of the data. Previous studies with "first generation" monoclonal antibody imaging reagents have shown that postinfusion blood concentrations can follow either patterns that resemble either one or two compartment models that reflect both the dose and the physiologic characteristic of the patient. Initial parameter estimates will be obtained using the JANA curve stripping programs (Statistical Consultants, Lexington Ky). These estimates will be applied to the appropriate model to best fit the time/concentration Monoclonal antibody data with respect to the duration of the infusion and the as well as the IV route of administration. The nonlinear models in PCNONLIN use LaGrangin techniques to reduce the total variance of appropriate parameters and yield the key pharmacokinetic parameters. These include half lifes for each compartment (e.g. alpha, and beta for 2 compartment models), the area under the plasma concentration versus time curve (AUC), the apparent volume of distribution, intercepts (e.g. A & B in a 2 compartment bolus injection). The time of distribution , total body clearance, and renal clearance are derived using urine excretion data. The clearance values will reflect the volume of blood and or urine from which the monoclonal antibody is removed per unit time (ml/min).

In patients with accessible tumors we will biopsy tumor 8 days after infusion of radiolabeled antibody to evaluate DPM.'s/gram tissue weight and immunohistochemical analysis of tumor antigen saturation by antibody.

10.2.5 Dosimetry calculations

Organ data will be obtained from the scans using region of interest analysis, attenuation correction of data, and the geometric mean of the anterior and posterior views of each large organ(liver,lungs, brain) and a single planar view for smaller organs and

measurable tumors. For marrow, it is assumed that 25% of the marrow volume is blood and that this represents the marrow to marrow exposure.

This process from region of interest analysis to MIRD dose calculations are performed using an integrated user interface on the SUN network. Image data is transferred to the optical disk and accessed using a SPARC station running *qsh* , an image handling toolkit. Once ROI's including normal organs, tumors, and background have been generated, the ROI statistics are corrected for attenuation and for background.The blood and organ radioactivity data will be examined using a computer program "S" designed to fit the data to a curve or curves. This program provides estimates of slopes and intercepts for each exponential component. The slopes and intercepts for the exponentials will be used to determine residence times and then the standard MIRD dosimetry formulations are applied to make dosimetric estimates.

Excretion rate plots (urinary excretion rate of radioactivity vs. time) will be examined in a manner similar to the analysis of the blood radioactivity data.

Finally, for measurable tumors, we will use the data obtained from imaging studies from the tumor site to estimate tumor dosimetry. Count density from the scans will be used to estimate change in tumor uptake over time. More helpful will be data obtained if and when accessible tumor is biopsied. This can be compared with scan data to develop dosimetric estimates.

10.3 Radioimmunoassay for BrE-3 antibody

Serum BrE-3 and Mc3 antibody is measured in a competition assay using iodinated-antibody, antibody tracer and cold antibody. This will be performed by Dr. Ceriani's laboratory at the Cancer Research Fund of Contra Costa

10.4 HPLC Analysis of Urine and Plasma

It is important to note that total radioactivity includes not only labeled monoclonal antibody, but also radioactivity that is circulating in the form of high molecular weight complexes (presumably antigen-antibody complexes). HPLC analysis will be used to determine the clearance of radioactivity versus the clearance of labeled antibody from the blood.

Analysis by HPLC will determine levels of serum and/or urine ^{111}In labeled BrE-3.

10 .5 Immunohistochemical analysis of biopsied tumor samples

Immunohistochemical staining for BrE-3 and Mc3 is performed under Dr. Howard Mizrachi's supervision with a trained histology technician performing all antibody staining. Tumor tissue will also be subject to routine histopathologic staining.

10.6 Immunoreactivity

Immunoreactivity testing will be performed after each radiolabelling procedure. The assay uses a single antibody concentration and increasing serial dilutions of the binding cell line or antigen coated beads to produce antigen excess.

10.7 Human Anti-antibody response

10.7.1 IgG

Anti-idiotype, anti-isotype, and cross reactivity with Mc3 will be quantitated using a " double antigen" technique(LoBuglio, Hybridoma, 1986). Latex beads coated with huBrE-3 will be incubated with 100 µl of serum and 1 µg of ^{125}I-antibody either huBrE-3,

huMc3 or human isotype matched (IgG1). Samples are incubated for 90 minutes and the beads are centrifuged through Percoll. Using the known specific activity of the radioiodinated antibody, the counts bound to the beads are converted to nanograms of of antibody/ml of serum. Serum samples obtained 1 week, one month and 3-6 months after antibody administration will be subjected to this assay to determine the anti-idiotypic and anti-isotypic response to the humanized BrE-3 and any cross-reactivity with humanized Mc3, the next antibody to be considered for clinical trials.

10.7.2 IgE
To quantitate patient serum anti-mouse IgE we will utilize the solid phase sandwich assay. Beads coated with BrE-3 antibody are mixed with the patient's serum. After incubation overnight, iodinated mouse anti-human IgE is added as a tracer. The beads are then washed and counted for the amount of tracer antibody bound to the beads.

11.0 Potential risks

11.1 Monoclonal antibody:

Murine BrE-3 has been administered to 18 patients to date. There has been no unexpected toxicity associated with BrE-3 in imaging or therapy trials. As with other murine monoclonal antibody trials in humans the overall toxicity with BrE-3 was mild and explainable in terms of allergic reactions. The allergic reactions included mild laryngospasm, occasional rash. One incidence of Grade 1 elevation of liver function tests occurred in a patient with marked liver involvement. There was no evidence of renal toxicity.

We will be administering the same or similar amounts of antibody.The radiolabeled antibody will be subject to quality control procedures including immunoreactivity testing, pyrogen testing (limulus amoebocyte lysate tests), and ITLC.
The antibody will be infused over a period of 2 hours with trained medical personnel in attendance. Vital signs will be monitored during the infusion and for an hour after. A baseline cardiopulmonary physical examination will be performed prior to and after administration of the radiolabeled antibody. An intravenous line which is placed for administration of the radiolabeled antibody will be kept in place. Antihistamines, epinephrine, and corticosteroids will be kept at hand. There is an emergency cart available in the Division of Nuclear Medicine. Patients with grade 1 or 2 toxicity may continue on study at the discretion of the investigator. In the event of more serious reactions, the antibody infusion will be stopped and treatment with subcutaneous epinephrine, intravenous steroids, respiratory assistance other resuscitative measures will be instituted. No further antibody will be administered.

Toxicity grading:

Toxicity will be graded according to NCI guidelines. (See Appendix).

Anti-antibody formation in patients who enter this protocol will be monitored as detailed above.

Adverse Drug Reaction Reporting

Report by phone to the Principal Investigator, FDA , NCI , IDB within 24 hours

a) All serious toxicity (Grade 3 and 4) which may be due to antibody administration

b) All fatal events

c) First occurrence of any toxicity regardless of grade other than Grade I fever . Written report to follow within 10 days.

Written reports to follow within 10 working days to :

Investigational Drug Branch

P.O. Box 30012

Bethesda, Maryland 20824

Radiation dosimetry:

Biodistribution of murine ^{111}In-BrE-3 has been studied in 15 patients with metastatic or recurrent breast cancer. It is not known how the humanization of will affect the biodistribution and pharmacokinetics of the radioimmunoconjugate although it is expected that the serum half-life will be affected. The following radiation dose estimations have been made for the murine ^{111}In-BrE-3:

Organ	Dose (rads/mCi) ^{111}In (mean± S.D.)
Kidneys	0.83±0.74
Liver	1.30±0.46
Lungs	0.95±0.46
Spleen	1.48±0.85
Whole Body	0.45±0.11
Total marrow	0.48±0.29
Blood to marrow	0.19±0.09
WB to marrow	0.29±0.22

Radiation monitoring/health safety

Gloves will be worn when handling samples of blood, urine, or other body fluids.

Laboratory specimens will be labeled with radioisotope labels.

There are no restrictions to visitors, including hospital personnel related to isotope administration. The radiation safety precautions and health safety precautions established by institutional radiation safety guidelines will be observed.

For outpatient administration, no special precautions are necessary in the patient's home. A history of contraceptive use or a negative pregnancy test will be required of all women of child-bearing potential entered in this protocol.

Venopuncture:

A total of 90 ml of whole blood will be drawn over a period of a week for pharmacokinetics. The early time point will be drawn through a small peripheral

intravenous line to minimize the number of needle sticks. In addition, approximately 25 ml will be required for routine CBC and chemistry as part of the initial evaluation and again for subsequent follow-up.

Venopuncture and blood drawing will be performed by experienced medical personnel. All laboratory specimens will be labeled with radioisotope labels. Efforts will be made to minimize the number of punctures necessary to obtain the requisite blood samples.

Confidentiality:
The records of the subjects entered into this study will be kept in a locked file. Beyond the study personnel, only representatives of the FDA or Coulter Immunology will have access to these files.

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Figure 1

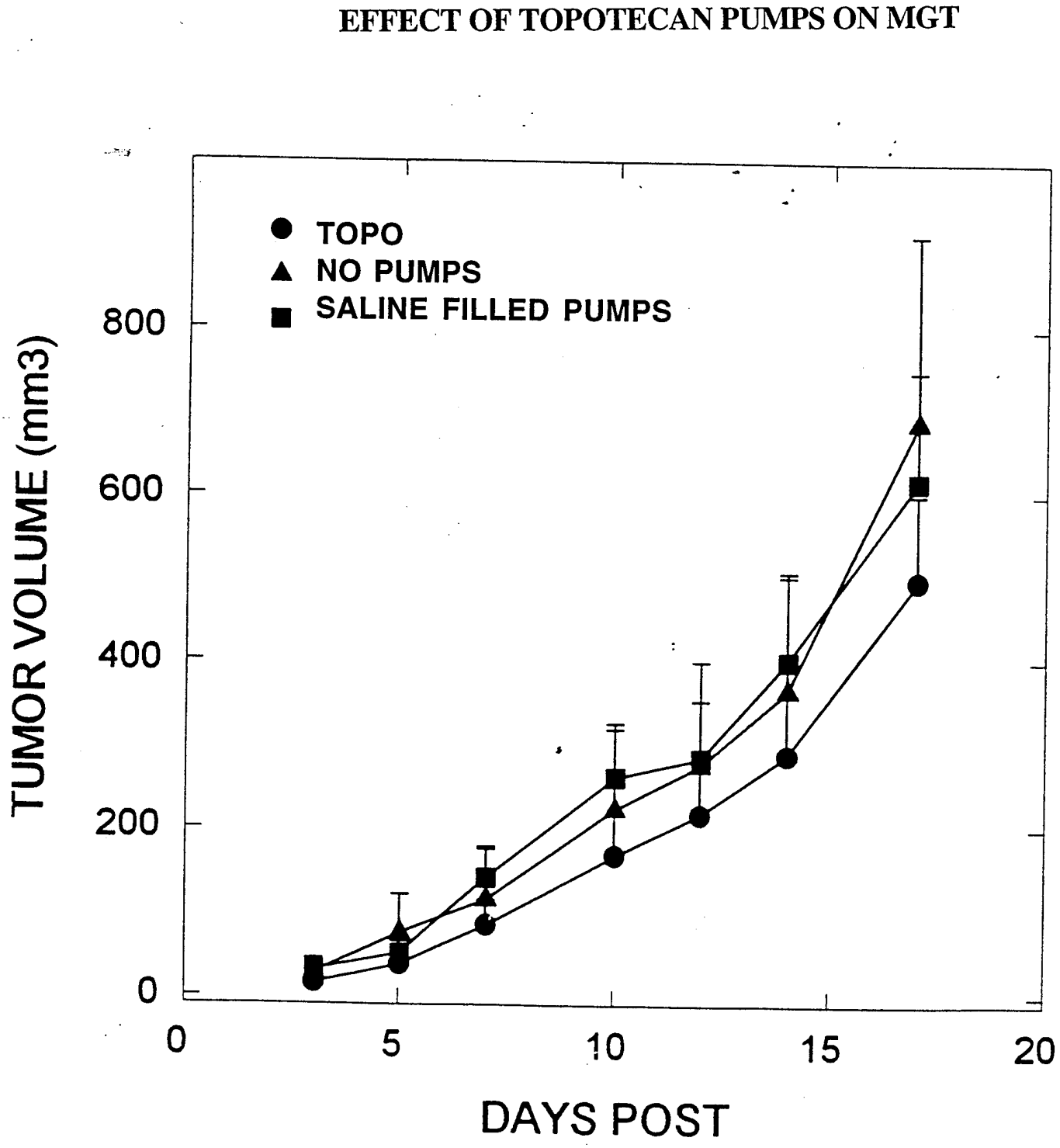
EFFECT OF TOPOTECAN PUMPS ON MGT
TOPO
NO PUMPS
SALINE FILLED PUMPS
TUMOR VOLUME (mm3)
800
600
400
200
0
0
5
10
15
20
DAYS POST

Figure 2

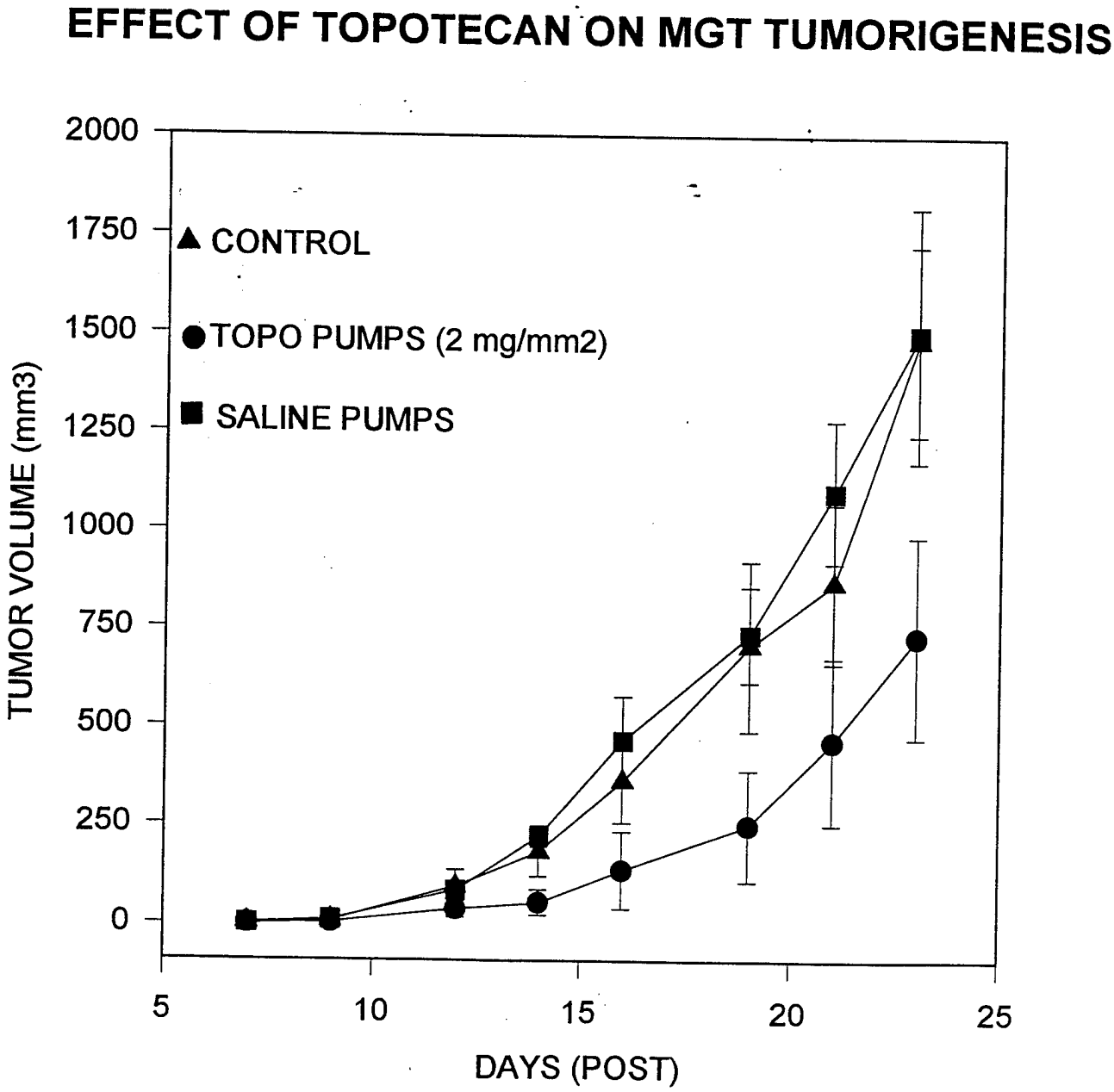
EFFECT OF TOPOTECAN ON MGT TUMORIGENESIS
2000
1750
1500
1250
1000
750
500
250
0
TUMOR VOLUME (mm3)
CONTROL
TOPO PUMPS (2 mg/mm2)
SALINE PUMPS
5
10
15
20
25
DAYS (POST)

Figure 3

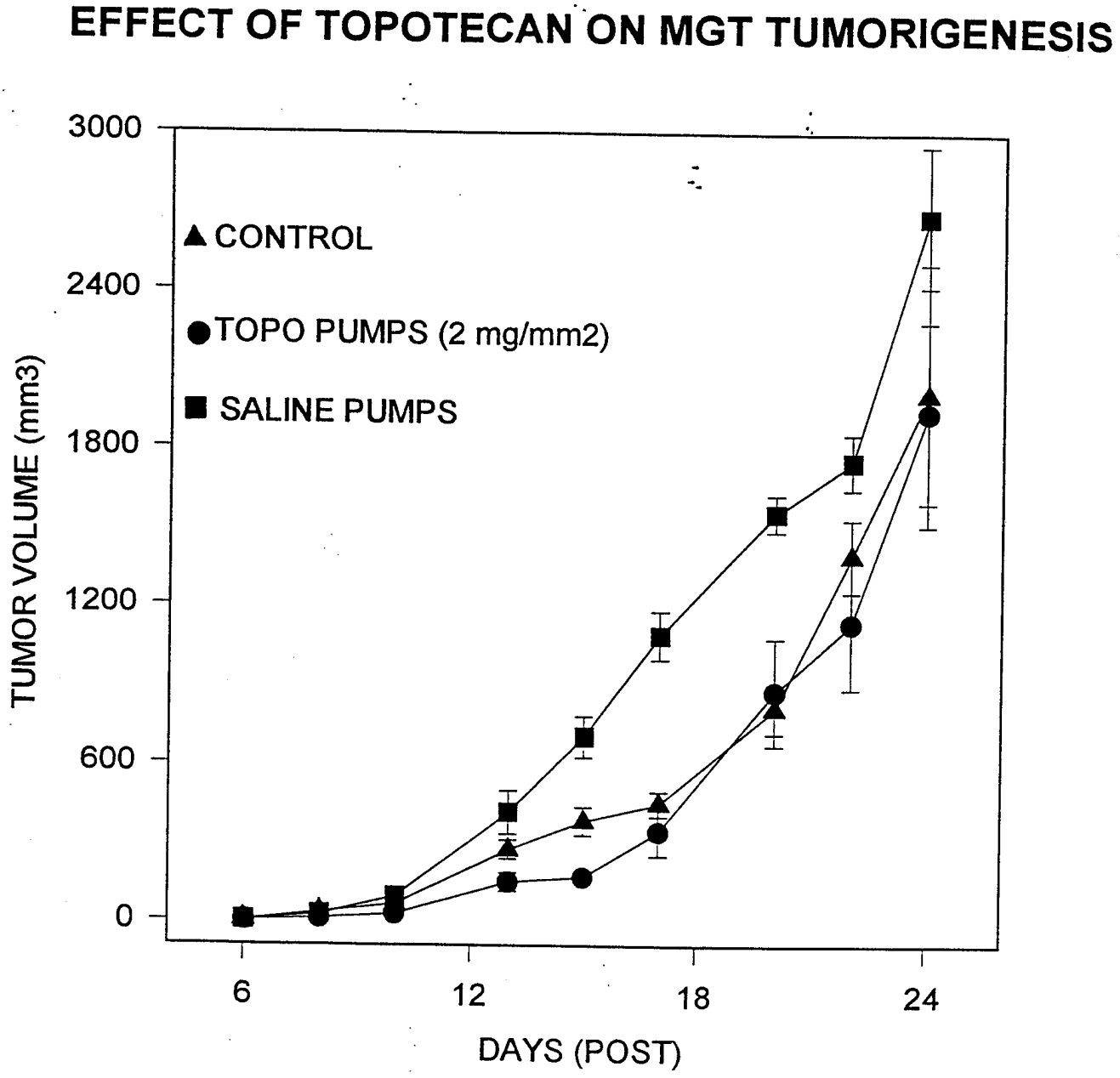
EFFECT OF TOPOTECAN ON MGT TUMORIGENESIS
3000
2400
1800
1200
600
0
TUMOR VOLUME (mm3)
▲ CONTROL
● TOPO PUMPS (2 mg/mm2)
■ SALINE PUMPS
6
12
18
24
DAYS (POST)

Figure 4

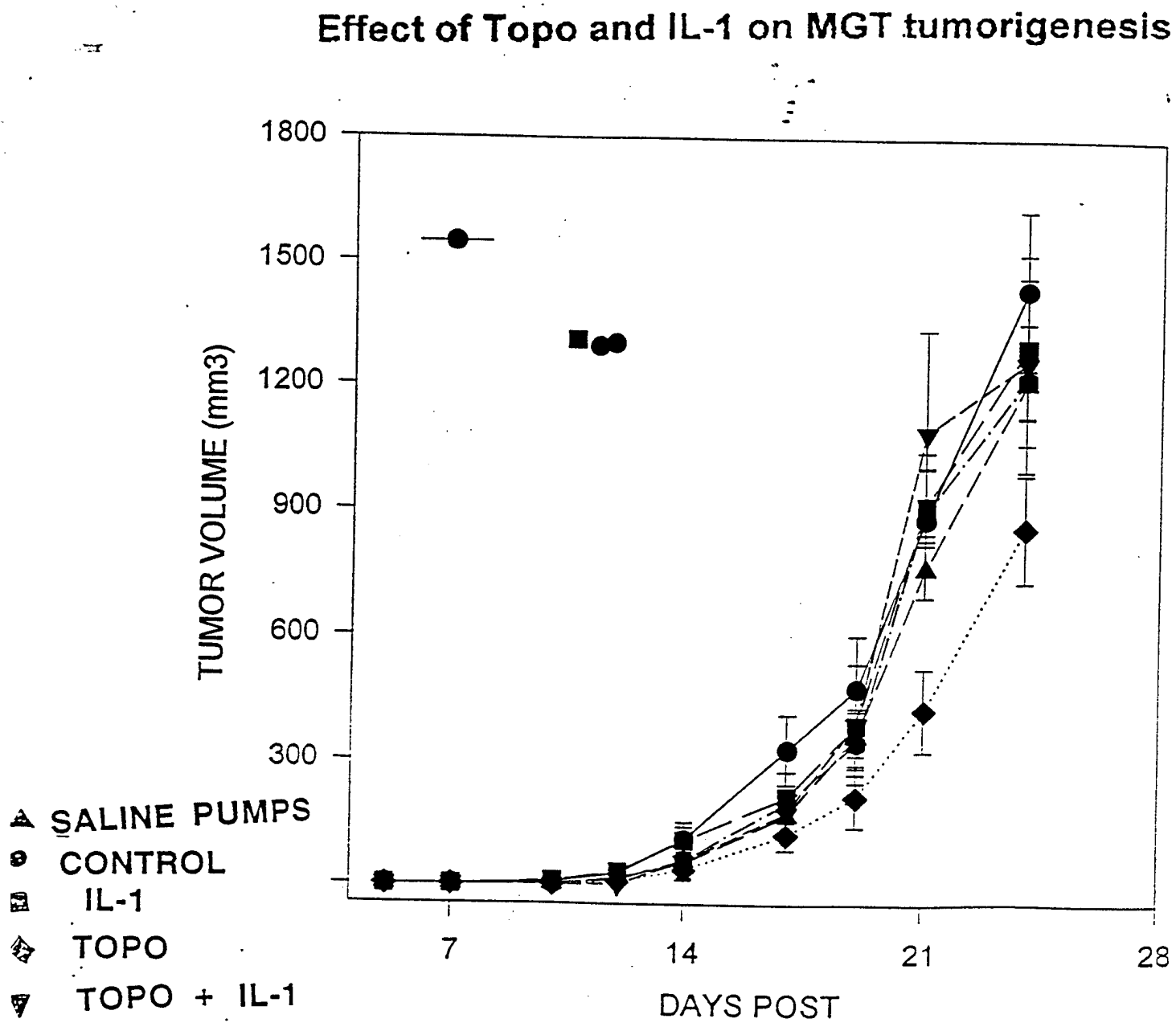
Effect of Topo and IL-1 on MGT tumorigenesis
1800
1500
1200
900
600
300
TUMOR VOLUME (mm3)
7
14
21
28
DAYS POST
SALINE PUMPS
CONTROL
IL-1
TOPO
TOPO + IL-1

Figure 5

Effect of Topo and IL-1 on MGT tumorigenesis