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Adriamycin custom-tailored immunoconjugates in the treatment of human malignancies

Robert K. Oldham, MD, Marvin Lewis, MD, Douglas W. Orr, MD, Barry Avner, PhD, Shuen-Kuei Liao, PhD, John R. Ogden, PhD, Belina Avner, BA, and Robert Birch, PhD

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Twenty-three patients with disseminated refractory malignancies each received a tailored combination of adriamycin-conjugated murine monoclonal antibodies. Tumors were typed using a panel of antibodies. Cocktails of up to six antibodies were selected based on binding greater than 80% of the malignant cells as tested by immunoperoxidase and flow cytometry. These monoclonal antibodies were then conjugated to Adriamycin and administered intravenously. Seventeen of 23 patients had reactions to the administration of immunoconjugates, but these were tolerable in all but two patients. Fever, chills, pruritis, and skin rash were by far the most common transitory reactions. All were well controlled with premedication. In several patients there was limited antigenic drift among various biopsies within the same patient over time. This observation confirms the necessity for the use of a cocktail of antibodies if one wishes to cover all tumor cells. Preliminary serologic evidence suggests that the development of an IgM antibody, which is specific against the mouse monoclonal antibody, has the specificity and sensitivity to predict clinical reactions. Selected patients were re-treated. One patient with chronic lymphocytic leukemia had re-treatment on three occasions and demonstrated regression of peripheral lymph nodes. Two patients with breast carcinoma had definite improvement in ulcerating skin lesions and two patients with tongue carcinoma had shrinkage of their lesions. In the course of the study free Adriamycin released from the monoclonal antibodies was discovered to be a limiting factor in the amount of antibody that could be administered. Up to 1 g of Adriamycin and up to 5 g of monoclonal antibody were administered. The limiting factor appeared to be a variable dissociation of active Adriamycin from the antibody that unpredictably caused hemopoietic depression. This study demonstrates the feasibility and reviews technical considerations in preparing immunoconjugate cocktails for patients with refractory malignancies. The major technical hurdle appears to be the selection of an effective conjugation method that can be used to optimally bind Adriamycin to monoclonal antibodies for targeted cancer therapy.

Keywords: Adriamycin; immunoconjugate cocktails; targeted cancer therapy

Introduction

Since Kohler and Milstein¹ provided the technique by which monoclonal antibodies could be produced in virtually unlimited quantities, there has been an explosion in the use of monoclonal antibodies in patients with malignancies. This paper introduces the concept of combination monoclonal antibodies, specifically tailored for individual patients, combined with Adriamycin.

The hypothesis that a combination of monoclonal antibodies would be necessary to cover virtually all cancer cells in a variety of sites and that each patient

would require an individually specified immunoconjugate dominated in this research. Single monoclonal antibodies have been demonstrated with immunoperoxidase tissue stains and radioisotopes to localize in areas of malignancy and to individual malignant cells.^{2,3} However, it is well known that cancer cells have a variety of antigens, which are not cancer-specific and which can vary within clusters of tumor cells both in one location and in distant metastatic sites (microheterogeneity). Tumor antigens may also vary during phases of tumor cell maturation. In addition, we have typed over 100 tumors from different patients, and quantitative differences are frequent. No two have demonstrated the same typing pattern (macroheterogeneity). Heterogeneity is basic to the thesis explored here. Thus, an attempt was made to identify a combination of antibodies, which could potentially recognize up to 100% of malignant cells within a variety of primary and metastatic sites. This was done by

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making a large number of monoclonal antibodies against tumors and then typing individual patient's tumor biopsies and demonstrating attachment to greater than 80% of the cells within the malignancy. To that end, cocktails of as many as six antibodies were administered to patients following conjugation with Adriamycin.

These antibodies were more than 95% pure, main-

tained immunoreactivity after conjugation, and were tested for safety in a variety of systems prior to administration to patients (*Figure 1*). This paper demonstrates the feasibility of treating patients with mixtures of monoclonal immunoconjugates and addresses technical considerations involved in the process. Observations of side effects, the re-treatment of patients subsequently with similar or identical antibodies, and the

Product Testing and Analysis

Nude Mouse Test



described elsewhere.⁸ Abbreviations: SDS-PAGE = sodium dodecyl sulfate polyacrilamide gel electrophoresis; HPLC = high performance liquid chromatography; FACS = fluorescent-activated cell sorter; PCFIA = Particle concentration fluorescence immunoassay.

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Papers

 Table 1. Monoclonal antibodies used in the present study

			Antigen
MoAb	lsotype	Immunogen	structure
BA-Br-1	lgG1	Membrane extract of	NDª
BA-Br-2	lgG1	Dispersed cells from	ND
BA-Br-3	lgG1	Membrane extract of breast carcinoma cell line CAMA-1	>300kD glyco- protein
BT-Br-4	lgG1	Dispersed cells from	ND
BA-Br-5	lgG1	Membrane extract of breast carcinoma tissue	220kD-400kD alvcoprotein
BT-Br-6	lgG1	Dispersed cells from breast carcinoma tissue	ND
BT-Co-1 ^b	lgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	29kD + 31kD protein
BT-Co-2	lgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	ND
BT-Co-3	lgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	ND
BT-Co-4	lgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	ND
BT-Co-5⁵	lgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	29kD + 31kD
BT-Co-6	lgG1	Dispersed cells from colon carcinoma grown as xenografts in nude mice	ND
BT-Me-3	lgG1	Dispersed cells from	ND
BT-Me-4	lgG1	Melanoma cell line CaCL 78-1	95kD–150kD glycoprotein
BT-Me-5	lgG2a	Melanoma cell line CaCL 78-1	p97-like (97kD) glycoprotein
BT-Me-7 BT-Me-8	lgG1 lgG1	Melanoma cell line BUR Melanoma cell line BUR	110kD protein 110kD + 40kD protein
BT-Ne-3	lgG1	Dispersed cells from hypernephroma tissue	ND

All antibodies are referenced to Liao et al.5

^a Not yet defined, although attempts were made to determine the molecular mass of antigen involved.

^b Based on epitope blocking and indirect immunoprecipitation experiments, BT-Co-1 and BT-Co-5 recognized different epitopes residing on the same or similar molecules.

biologic effects and tumor localization of the antibodies, as well as the efficacy of this treatent, are presented.

Materials and Methods

Patient selection

This clinical trial was carried out in Williamson Medical Center after approval by the Investigational Re-

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view Board of that institution. Patients were referred primarily by oncologists after failure of standard modalities of treatment. Each patient was initially seen by a medical oncologist who reviewed the history and medical records, confirmed the lack of standard therapeutic options available to the patient, informed the patient of the experimental nature of the study, and had a full discussion with each patient of the strategy involved in this therapy and of other experimental therapeutic options available. After a determination of suitability for the study and informed consent, tissue samples were obtained by biopsy. All typing was done on frozen tissue, either directly or on tissue that had been expanded by a xenograft in nude mice or by tissue culture propagation. Antibody selection was by immunoperoxidase and flow cytometry as described in detail elsewhere.^{4,5} A minimal period of 45 days was necessary for tissue typing and preparation of sufficient quantities of immunoconjugate for treatment. A typical regimen consists of 3 days for typing the tissue with a panel of monoclonal antibodies, a period of 4 weeks for production of sufficient quantities of the individual antibodies followed by conjugation of Adriamycin, and extensive safety testing over a final 3 weeks (Figure 1). Thus, within 3 months patients were seen and treated with a tailored combination of antibodies conjugated to Adriamycin.

Immunoconjugate preparations dissolved in normal saline were given on a Monday, Wednesday, and Friday over a period of 1 to 5 hours. Total amounts of immunoconjugate were generally given over a 2- to 3week period. An initial test dose of 10 mg of Adriamycin bound to antibody was given. The dose was then quickly escalated depending on the phase of the study. Early in the investigation total Adriamycin doses were kept below 300 mg. Near the end of the investigation, antibody amounts were escalated to try to give as much as 1 g of Adriamycin and 3 to 5 g of antibody over a period of 2 to 3 weeks. A registered nurse was always available during administration, and patients were premedicated with acetaminophen and diphenhydramine for fevers, meperidine for rigors, and epinephrine in four patients for significant allergic reactions.

Antibody selection and preparation

Immunization of mice and preparation of hybridomas are described elswewhere.^{4,5} Over 100 antibodies were available for tissue typing, and we selected 28 for the standard panel. Seven of these were acquired elsewhere and 21 were produced in the laboratories of Biotherapeutics. Five of these originated from immunization with breast cancers, 11 from melanomas, 3 from adenocarcinomas of the kidney, 2 from an islet cell carcinoma of the pancreas, and 7 from colon carcinomas.⁴ The majority of the antibodies were IgG₁ with the exception of two IgG₂'s (melanoma) and five IgG₃'s (colon carcinomas). *Table 1* illustrates the characteristics of 18 antibodies from the panel used in this clinical study.

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