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Individually specified drug immunoconjugates in cancer treatment

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ABSTRACT: Forty-three patients with disseminated refractory malignancies each received an individually-specified combination of either Adriamycin (24 patients) or mitomycin-C (19 patients) conjugated murine monoclonal antibodies. Tumors were typed using a panel of antibodies with both immunohistochemistry and flow cytometry. Cocktails of up to six antibodies were selected based on binding greater than 80% of the malignant cells in the biopsy specimen. These monoclonal antibody cocktails were drug conjugated and administered intravenously.

Seventeen out of twenty-four patients had reactions to the administration of Adriamycin 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 it was demonstrated that there was limited antigenic drift among various biopsies within the same patient over time. Up to 1 gram of Adriamycin and up to 5 grams of monoclonal antibody were administered. The limiting factor appeared to be a variable dissociation of active Adriamycin from the antibody which unpredictably caused hemopoietic depression.

Similar findings were noted in 19 patients with mitomycin-C conjugates. Thrombocytopenia at a 60mg dose of mitomycin-C in this schedule was dose limiting. Preliminary serological 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. These antibodies were quantitatively less in mitomycin-C patients.

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. No responses were seen with mitomycin-C conjugates but binding was noted to tumors and colon with likely drug induced colitis seen after colon binding.

This study demonstrates the feasibility and illustrates technical considerations in preparing drug immunoconjugate cocktails for patients with refractory malignancies.

Cocktail formulation and antibody delivery was accomplished. The major technical hurdle appears to be the selection of effective conjugation methods that can be used to optimally bind drugs to monoclonal antibodies for targeted cancer therapy. (Int J Biol Markers 1989; 4: 65-77)

KEY WORDS: Monoclonal antibodies, Drug immunoconjugate cocktails, Targeted cancer therapy

INTRODUCTION

Since Kohler and Milstein (1) provided the seminal technology, there has been an explosion in the use of monoclonal antibodies in patients with malignancies. This paper describes our series of patient treated with combination monoclonal antibodies, specifically tailored for individual patients, combined with Adriamycin or mitomycin-C.

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 this research. Single monoclonal antibodies 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. Antigens can vary within patients in clusters of tumor cells both by location and over time (microheterogeneity). Tumor

antigens may also vary during phases of tumor cell maturation. In addition, we have typed tumors from more than 150 patients and quantitative differences are the rule. No two have demonstrated precisely the same typing pattern (macroheterogeneity). Thus, an attempt has been made to identify combinations of antibodies which could potentially recognize up to 100% of malignant cells within a variety of primary and metastatic sites. This was done by making a large number of monoclonal antibodies against freshly dispersed cells, xenograft cells or cell lines recently derived from biopsies of tumors and then typing the individual patient's tumor biopsies with these antibodies. Cocktails were specified to bind greater than 80% of the cells within the malignancy. To that end, preparation of as many as six antibodies were administered to patients following drug conjugation.

These antibodies were usually greater than 95% pure, maintained immunoreactivity after conjugation and were tested for safety in a variety of systems prior to administration to patients. This paper demonstrates the feasibility of treating patients with mixtures of monoclonal immunoconjugates and addresses technical considerations involved in the process. Observations on side effects, the retreatment of patients subsequently with similar or identical antibodies, the biological effects and tumor localization of the antibodies and the efficacy were recorded.

MATERIALS AND METHODS

Patient Selection

Twenty males and twenty-three females participated in this trial. The most common cancers were breast (14), lung (3), colorectal (5), ovary (3), renal (2), and prostate (3).

A variety of other cancer types were included as shown in Table 1. Selected characteristics of the treated patients are shown in Table 2.

This clinical trial was carried out in Williamson Medical Center, after approval by the Investigational Review Board. Patients were referred primarily by oncologists after failure of standard modalities. Each patient was initially seen by a medical oncologist who reviewed the history and medical records, confirmed the failure of standard therapeutic options and, informed the patient of the experimental nature of the study. Each patient understood the strategy involved in this therapy and the other experimental therapeutic options available. After a determination of a 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 which had been expanded by a xenograft in nude mice or by tissue culture propagation. Antibody selection was by immunoperoxidase and flow cytometry as described

TABLE 1 - DISEASE CATEGORIES

Tumor type	No. patients treated with Adriamycin immunoconjugates	No. patients treated with Mitomycin-C immunoconjugates	Total
Breast CA	10[a]	4[a]	14[a]
Carcinosarcoma	0	1	1
Cholangiosarcoma	1	0	1
Colo-rectum CA	2	3	5
Hepatoma	0	1	1
Islet Cell CA	1	0	1
Leiomyosarcoma	1	1	2
Lung CA	2	1	3
Lymphoma (CLL)	1	0	1
Ovarian	1	2	3
Parotid	1	0	1
Prostate CA	1	2	3
Renal Cell CA	1	1	2
Schwannoma	0	1	1
Squamous Cell CA (tongue, mouth, penis)	3	2	4
	24	19	43

[a] Note that one patient with breast infiltrating ductal carcinoma first received Adriamycin immunoconjugate and six months later Mitomycin-C immunoconjugate therapy. Thus, the total number of different patients with breast carcinoma is 13 instead of 14.

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 consisted of three days to type with a panel of monoclonal antibodies, four weeks for production of sufficient quantities of the individual antibodies followed by conjugation of drug and extensive safety testing over a final three weeks. Thus, within three months, patients were seen and treated with an individually tailored combination of drug conjugate antibodies.

Immunoconjugate preparations dissolved in normal saline were given over a period of one to five hours on a Monday, Wednesday and Friday. The total amount of immunoconjugate was given over a two to three week period. An initial test dose based on 5-10 mg of drug bound to antibody was given. The dose was then quickly escalated depending on the phase of the study. Toward the end of the investigation, antibody amounts were escalated to try to give as much as one gram of Adriamycin and 60 mg of mitomycin C conjugated to 3-5 grams of antibody over a period of 2-3 weeks. A registered nurse was always available during administration and patients were pre-medicated 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 elsewhere (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 biotherapeutics' laboratory. Five of these originated from immunization with breast cancers, eleven from melanomas, three from adenocarcinomas of the kidney, two from an islet cell carcinoma of the pancreas, and seven from colon carcinomas (4).

The majority of the antibodies were IgG_1 with the

TABLE 2 - MONOCLONAL ANTIBODIES USED IN THE PRESENT STUDY[a]

MoAb	Isotype	Immunogen	Antigen structure
BA-Br-1	IgG1	Membrane extract of breast carcinoma tissue	ND[b]
BA-Br-2	IgG1	Dispersed cells from breast carcinoma tissue	ND
BA-Br-3	IgG1	Membrane extract of breast carcinoma cell line CAMA-1	> 300kD glycoprotein
BT-Br-4	IgG1	Dispersed cells from breast carcinoma tissue	ND
BA-Br-5	IgG1	Membrane extract of breast carcinoma tissue	220kD - 400kD glycoprotein
BT-Br-6	IgG1	Dispersed cells from breast carcinoma tissue	ND
BT-Co-1[c]	IgG3	Dispersed cells from colon carcinoma grown as xenografts in nude mice	29kD + 31kD protein
BT-Co-2	IgG3	"	ND
BT-Co-3	IgG3	"	ND
BT-Co-4	IgG3	"	ND
BT-Co-5[c]	IgG3	"	29kD + 31kD
BT-Co-6	IgG3	"	ND
BT-Me-3	IgG1	Dispersed cells from melanoma tissue	ND
BT-Me-4	IgG1	Melanoma cell line CaCL 78-1	95kD-150kD glycoprotein
BT-Me-5	IgG2[a]	Melanoma cell line CaCL 78-1	p97-like (97kD) glycoprotein
BT-Me-7	IgG1	Melanoma cell line BUR	110kD protein
BT-Me-8	IgG1	Melanoma cell line BUR	110kD + 40kD
BA-Me-10	IgG2[a]	Melanoma cell line	280kD + >400kD
BA-Me-11	IgG1	"	"

[a] All antibodies are referenced as to source in reference 5
[b] Not yet defined, although attempts were made to determine the molecular mass of antigen involved
[c] 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.

exception of two IgG_2's (melanoma) and five IgG_3's (colon carcinomas). Table 3 illustrates the characteristics of 19 antibodies from the panel used in this clinical study.

The initial selection of antibodies was done by immunohistochemical phenotyping (6-9). This immunoperoxidase based system is fully described elsewhere (5). The selection of antibodies was based on a grading system of 1 to 4+ which included judgements on the intensity of staining as well as the distribution of staining and the specific characteristics of the staining. The variation in grading between observers was less than 10%. The selection by the same observer was reproducible over 90% of the time. Staining patterns varied from homogeneous staining of membrane and cytoplasm to patchwork staining of given tumor areas leaving adjacent tumor areas virtually unstained, to scattered reactivity of tumor cells in a "sea" of non-reactive tumor cells (5).

Selections of antibodies were made which encompassed considerations of intensity, distribution, and patterns of staining. Positive controls included anti-HLA; negative controls included nonspecific random mouse immunoglobulins. All results were scored independently by two scientists.

Frozen tissue specimens (from fresh or cryopreserved primary or metastatic tumors) were assessed for binding of 19 murine monoclonal antibodies from our panel. One-micron thick sections of frozen tissue embedded in OCT compound (polyvinyl alcohol, benzalkonium chloride, polyethylene glycol, d.H_20; Miles Labs, Elkhart, Indiana), were cut, placed on gelatin-coated slides and evaluated by means of the avidin-biotin-peroxidase complex technique (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Enzymatically or mechanically, disaggregated tumor cells (when available) were also subjected to flow-cytometric analysis to determine cell percentage and intensity of antibody binding.

The use of the HLE-1 antibody, which binds to leucocytes, permitted the correction for any white blood cell contamination. Propidium iodide exclusion staining excluded non-viable cells. Simultaneous antibody exposures provided comparisons of various antibody combinations to define possible additive or interfering interactions.

TABLE 3 - IMMUNOHISTOCHEMICAL RESULTS OF METASTATIC MELANOMA LESIONS REMOVED AT VARIOUS TIMES FROM PATIENT BUR

	Occipital LN met 01/23/86	Tissue culture cells from LN met 01/23/86	Mediastinal LN met 05/05/86	Neck LN met 04/27/87	Supraclavicular LN met 06/15/87	Femoral LN met 06/15/87	Brain met 09/28/87
ANTI-MELANOMA							
BT-Me-7	4+	1+	4+	4+	4+	4+	4+
BT-Me-8	4+	2+	4+	4+	4+	4+	4+
BT-Me-3	4+	4+	—	—	—	—	—
BT-Me-4	4+	4+	2+	1+	4+	4+	4+
BT-Me-5	—	—	—	—	1+	2+	2+
ANTI-BREAST CA							
BA-Br-1	—	—	—	—	—	—	—
BA-Br-3	—	—	—	—	1+	1+	2+
BA-Br-4	3+	—	—	—	3+	3+	2+
BA-Br-5	—	—	—	—	—	—	—
BA-Br-6	—	—	4+	4+	4+	4+	2+
R-11	—	—	4+	4+	4+	4+	1+
R-13	4+	1+	3+	3+	4+	4+	NE
ANTI-COLON CA							
CO-Co-1	—	—	—	—	—	—	—
BT-Co-2	—	—	—	—	—	—	—
BT-Co-3	—	—	—	—	—	—	—
BT-Co-4	—	—	—	—	—	—	—
BT-Co-5	—	—	—	—	—	—	—
BR-Co-6	—	NT	—	—	—	—	—
BR-Co-7	—	NT	—	—	—	—	—
BR-Co-8	—	NT	—	—	—	—	—
BR-Co-9	—	NT	—	—	—	—	—
ANTI-RENAL CA							
BT-Ne-3	1+	1+	—	—	—	—	—

NE = Not available because tissue section was washed off
NT = Not tested

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