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EGFR is a transmembrane tyrosine kinase that binds a variety of ligands including EGF, transforming growth factor-α, and amphiregulin. Ligand binding induces activation of the tyrosine kinase leading to growth stimulation, but also perhaps to inhibition of apoptosis and other proliferative phenomena. The bioactivity of monoclonal antibodies (Mabs) against EGFR that we have produced is well documented. The human:murine chimeric version of Mab 225 (HC Mab 225) has been produced by ImClone Systems. These Mabs inhibit the growth of tumors expressing EGFR and synergize with either doxorubicin or paclitaxel against well-established tumor xenografts. Preliminary clinical trials with murine anti-EGFR Mabs conducted by our group have shown that their administration is safe and that plasma levels of Mab sufficient to saturate receptors can be achieved. This report present investigation describes the safety, feasibility, and noncomparative efficacy of chemotherapy plus Mab in the treatment of patients with metastatic breast cancer who have not received extensive prior chemotherapy for their advanced disease. After thorough review of the preclinical data , we studied the combination of paclitaxel and HC Mab 225. This decision was also based on considerations of patient availability, sincedoxorubicin is now widely used in the adjuvant setting.

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FOREWORD

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Andrew Seidel 2/2/98

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INTRODUCTION

EGFR is a transmembrane tyrosine kinase that binds a variety of ligands including EGF, transforming growth factor-α, and amphiregulin. Ligand binding induces activation of the tyrosine kinase leading to growth stimulation, but also perhaps to inhibition of apoptosis and other proliferative phenomena. The bioactivity of monoclonal antibodies (Mabs) against EGFR that we have produced is well documented (1). The human:murine chimeric version of Mab 225 (HC Mab 225) has been produced by ImClone Systems. These Mabs inhibit the growth of tumors expressing EGFR and synergize with either doxorubicin or paclitaxel against well-established tumor xenografts (2-5). Preliminary clinical trials with murine anti-EGFR Mabs conducted by our group have shown that their administration is safe and that plasma levels of Mab sufficient to saturate receptors can be achieved (6,7). The present investigation is to determine the safety, feasibility, and noncomparative efficacy of chemotherapy plus Mab in the treatment of patients with metastatic breast cancer who have not received extensive prior chemotherapy for their advanced disease. After thorough review of the preclinical data (Appendix A), we elected to first proceed with the study of paclitaxel and anti-EGFR Mabs. This decision was also based on considerations of patient availability, since doxorubicin is now widely used in the adjuvant setting.

BODY

The selection of patients for these clinical trials required that an efficient mechanism be established for the identification of potential candidates based on tumoral immunohistochemical expression of EGFR. All members of the Breast Cancer Medicine Service were involved in the procurement of paraffin-embedded tumor tissue, which was directed under the supervision of a designated research assistant to the laboratory of Dr. Peter Paul Rosen, in our Department of Pathology. Immunohistochemistry results were compiled in a computer database, and reports generated weekly for review by the Principal Investigator, in order to allow for timely identification of possible protocol candidates. To date, 13.4% of all breast carcinoma specimens have stained positively for EGFR.

The construction of a feasible phase I/II trial required the determination of the safety and pharmacokinetics of multiple administrations of the drug HC Mab 225. We therefore first performed an open-label dose-escalation study of four weekly infusions at the dose levels of 5 (n=1), 20 (n=2), 50 (n=1), and 100 mg/m2 (n=3) per week in patients with histologically documented advanced tumors over-expressing EGFR by immunohistochemistry (12 patients were enrolled at MSKCC, with 5 patients accrued at other centers). The median age was 60 years, and several tumor types were represented, including breast cancer. Only one patient experienced grade 3 toxicity, an episode of "aseptic meningitis" perhaps unrelated to drug administration; one grade 2 allergic reaction was noted. All other toxicities were grade 1, and included: acneiform rash (3 episodes), fatigue (2), hot flashes (1), anorexia (1), chills (1), flu-like symptoms (1), thrombocytopenia (1), stomatitis (1), elevation of alkaline phosphatase (1), and creatinine (1).

HC Mab 225 pharmacokinetics was assessed by the BIAcore (suface plasmon resonance) assay on serum samples drawn at 1/24, 3/24, 6/24, 1,2,5,8,15,22,26,and 28 days post-infusion. We sought to obtain a serum level of at least 20 nM, as preclinical evidence suggested that this would result in occupancy of a high proportion of receptors in target tissues (the notion of "saturation of receptors" does not apply since EGFR is widely distributed in normal organs). At the 50 mg/m2 dose level, the mean concentration of drug was greater than 20 nM for > 1 day. At 100 mg/m2 the mean concentration of drug was greater than 20 nM for >7 days, allowing for drug accumulation. Saturation of clearance was not seen. Hence we became confident that a trial employing weekly administrations of 100 mg/m2 doses of drug would be adequate to elicit the desired biological effects.

Our phase I/II trial of the combination of HC Mab 225 and paclitaxel was open to patients with histologically documented metastatic breast cancer, regardless of immunophenotypic expression of EGFR, with bidimensionally measurable disease, normal hematologic and organ function, adequate performance status, no prior taxane, and < 2 prior chemotherapy regimens for

metastatic disease. The study was designed to accrue 3 patients each at the following initial and subsequent doses in mg/m2 of HC Mab 225: 50/50, 100/100, 200/100, 400/100, with subsequent doses to be specified on the basis of the pharmacokinetic analysis. Paclitaxel was to be given at the conventional dose of 175 mg/m2 as a 3 hour infusion each 3 weeks, with standard premedications.

We initially treated 9 patients with the combination of weekly HC Mab 225 with "standard" paclitaxel dosing at 175 mg/m^2 via 3-hour infusion every 3 weeks (Appendix B). During this time, we observed a significant occurrence of moderate to severe skin toxicity: an erythematous follicular eruption of the face, trunk, and upper extremities of grade 2-3 severity in 4/9 evaluable patients (selected photographs of skin reactions in Appendix C). Skin biopsies of these lesions in 3 cases has demonstrated superficial folliculitis, with adjacent edema and mixed neutrophil and eosinophil, or pure neutrophil-rich inflammatory cell infiltrate with scattered histiocytes. Immunohistochemistry for EGFR in these skin biopsies revealed normal EGFR expression within keratinocytes. Of these 9 patients who were evaluable for antitumor response, two have shown minor tumor regression, but one of these had to discontinue treatment because of dermatologic toxicity.

These data suggest synergistic biologic activity between HC Mab 225 and paclitaxel, but in the skin. We were not able to assess if this synergy extends to the tumor, because the toxicity observed precluded adequate evaluation, both in terms of number of patients accrued and duration of follow-up. However, no early indications of synergistic anticancer benefit had been observed. While several patients consented to undergo skin biopsies in an effort to better elucidate the nature of the dermatologic reactions encountered, unfortunately no patient to date has consented to allow the perfomance of serial biopsies of accessible tumor tissue. Thus, we have, to date, been unable to perform the planned studies of EGFR and TGF-alpha regulation, EGFR phosphorylation, and apoptosis outlined in our original statement of work.

After careful examination of potential strategies to maximize synergistic antitumor effects, while minimizing potential for cutaneuos phenomonena, we chose to modify the administration schedule for these two agents. Given that paclitaxel may contribute to the toxicity (similarly frequent and severe cutaneous reactions with HC Mab 225 in combination with other cytotoxic agents, e.g. doxorubicin and cisplatin, have *not* been noted in other clinical trials in other solid tumors), we reassessed the Mab given weekly with an alternate schedule of paclitaxel -- paclitaxel was administered weekly at 80 mg/m^2 as a 1-hr infusion to the next three patients (with weekly HC Mab 225). In patients with ovarian carcinoma we have determined that this dose and schedule of paclitaxel is safe and effective (8). We have also completed a phase II and pharmacologic study of paclitaxel at 100 mg/m^2 in patients with minimally pretreated metastatic breast cancer, with a final response rate of 53.3% (95% C.I. 40-66%), including 3 complete remissions (9). Hence, we combined HC Mab 225 with an active regimen of paclitaxel, but with one that achieves lower peak plasma levels because of the lower total dose per administration, and additionally has been reported to cause less alopecia (follicle effect). The potential differences in paclitaxel's pharmacology (as a 175 mg/m^2/week 3-hour infusion every 3 weeks vs. as an 80 mg/m^2 infusion once weekly), and paclitaxel scheduling change on hair follicles motivated us to study this

alternative drug delivery plan. Indeed, if an important intratumoral synergistic effect is expected, one might expect this to be enhanced by weekly co-administration of both agents.

Clinical Protocol Update: Since October 1997 we have treated 3 patients with weekly coadministration of paclitaxel and HC Mab 225. All three patients experienced folliculitis: the first patient's was of grade 1 severity (protocol treatment was discontinued after 6 weeks due to disease progression), the second was of grade 3 severity and required discontinuation of protocol therapy despite a minor response after 6 weeks, and the third patient has experienced a grade 2 follicular skin reaction, presently stable at week 5 after early initiation of topical corticosteroid and systemic antibiotic (oral erythromycin) treatment. All three patients have been evaluated by a dermatologist, and skin reactions photographed (Appendix C).

CONCLUSIONS

The enhancement of chemotherapeutic agents with novel agents that perturb signal transduction pathways may allow for therapy with a higher therapeutic index due to variable effects on malignant and non-malignant cells, likely due to tissue-specific differences in cell cycle checkpoint regulation (10). However, based on our clinical experience to date with the HC Mab C225 directed against EGFR (HER1) and paclitaxel combinations, we find it highly unlikely that it will be possible to uncouple the synergistic effect observed in skin from the potential synergy expected in breast cancer.

During the course of these investigations, in separate work, we demonstrated the clinical activity of the humanized monoclonal antibody directed against the related tyrosine kinase growth factor receptor, HER2/*neu* (rhuMab HER2)(11). A large, multicenter randomized clinical trial expected to be reported at the upcoming Annual Meeting of the American Society of Clinical Oncology this May will decribe important *clinical synergy* for the combination of paclitaxel and rhuMab (personal communication, Dr. Larry Norton, 12/97). Given this translation of preclinical synergy, and the obstacles we have encountered in combining paclitaxel with HC Mab 225 (*vide supra*) we have refocused our laboratory investigations in an effort to define the mechanisms of this apparent synergy, and to examine the potential synergy of other agents that act downstream in the signal transduction cascade, such as farnesyl transferase inhibitors. These investigations, and other related laboratory and correlative science investigation is described in greater detail in a revised statement of work (attached, Appendix C), to be supported by residual funds from the this grant, given the findings reported in the Body of this report.

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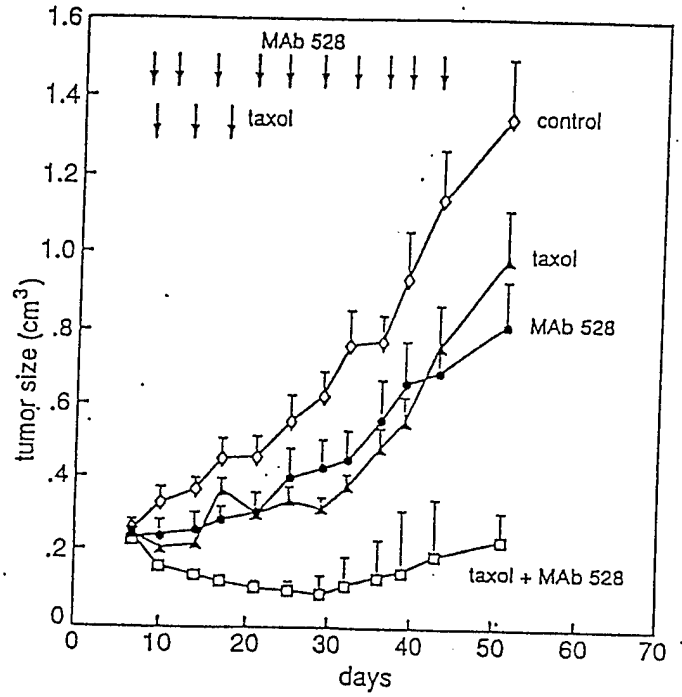
APPENDIX A

C225 Anti-EGFR MAb + Paclitaxel in MDA-468 Breast Carcinoma Cells



Antitumor activity of MAb 528 in combination with paclitaxel (taxol) on well established MDA-468 breast adenocarcinoma xenografts. Treatment was started when tumors reached a mean size of 0.2 cm^3 . A total of 8 mice were treated in the combination group. Results are given in mean tumor size ± SE. Paclitaxel 10 mg/kg i.v. was given on days 1, 4 and 9 of treatment and MAb 528 (2mg) was given i.p. on day 1 of treatment and twice a week thereafter for a total of 10 doses. Treatment with either doxorubicin alone or MAb alone partially inhibited growth. Paclitaxel in combination with MAb 528 resulted in a marked antitumor effect. Arrows show days on which treatment was administered.

APPENDIX B

Results of HC Mab 225 plus Paclitaxel in Stage IV Breast Cancer

#	EGFR	Dose	Response	Off-Study	Skin Toxicity (worst grade)
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"Standard" Paclitaxel + HC Mab 225:					
1	(+)	50/50	MR	PD 3 cycles	0
2	(+)	50/50	MR	SD 3 cycles	2
3	(+)	50/50	PD	PD 3 cycles	1
4	(-)	100/100	PD	PD 1 cycle	0
5	(+)	100/100	PD	PD during 1	2*
6	(-)	100/100	PD	PD after 1	3*
7	(-)	100/100	SD	PD after 1	1
8	(-)	100/100	SD	TOX	3*
9	(+)	100/100	SD	PD 2 cycles	1
Weekly Paclitaxel + HC Mab 225:					
10	(+)	100/100	PD	PD 1 cycle	1
11	(+)	100/100	MR	TOX	3
12	(+)	100/100	TE	TE	2#

SD = Stable Disease
MR = Minor Reponse
PD = Progressive Disease
TE = Too Early to Assess Response

TOX= Patient Discontinued Protocol Therapy Due to Toxicity (Skin)

* Skin Biopsy Obtained # Patient Actively Receiving Protocol Therapy

APPENDIX C

PHOTOGRAPHS OF ENCOUNTERED DERMATOLOGIC TOXICITY DURING TREATMENT WITH PACLITAXEL + HC Mab 2225