Phase I Clinical Trial of the Chimeric Monoclonal Antibody (c30.6) in Patients with Metastatic Colorectal Cancer

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ABSTRACT

The murine antibody 30.6 recognizes an antigen that is expressed on a high proportion of colorectal carcinomas and their metastases. We report the results of single-dose escalation studies of the chimeric 30.6 (c30.6) monoclonal antibody in metastatic colorectal cancer, to evaluate its safety, pharmacokinetics, and biodistribution.

Recombinant c30.6 (IgG1κ) antibody was secreted from Chinese hamster ovary cells and purified by a multi-step chromatography process. Seventeen patients with metastatic colorectal cancer were enrolled in this dose escalation study. The first four patients were treated with 3 mg of 125I-labeled c30.6, whereas the next 13 received a single dose of unlabeled antibody (maximum dose, 50 mg/m²).

The most frequent side effect was a novel syndrome of severe burning and erythema of the face, chest, neck, ears, palms, soles, and genitalia. The frequency of this syndrome was markedly reduced in those patients premedicated with high doses of histamine receptor 1 and histamine receptor 2 blockers. Other side effects were mild and predictable. Biodistribution studies showed a rapid and intensive hepatic uptake. At the 50 mg/m² level the half-life and maximum serum concentration were 81 ± 15 h and 7.9 µg/ml, respectively. One patient developed a low-level human anti-c30.6 response. Tumor response was assessed by computed tomography, positron emission tomography scanning, and serial carcinoembryonic antigen measurements. There were no partial responses, although positron emission tomography scanning demonstrated some reduction in tumor activity in three individuals.

The chimerized c30.6 antibody is not immunogenic in humans and appears worthy of further study. It does, however, produce a unique profile of side effects that can be well controlled with premedication.

INTRODUCTION

Over the last few years, a number of clinical studies have provided evidence that monoclonal antibodies are of value in the treatment of a variety of cancers, including breast, lymphoma and colorectal tumors (1–3). Although adjuvant chemotherapy modestly improves the disease-free and overall survival of patients with Dukes stage C colorectal cancer, there is a need for new adjuvant therapies that have both improved efficacy and fewer side effects. In this regard, the proven efficacy of adjuvant 17-1A therapy in a small group of patients with Dukes stage C colon cancer has shown that antibodies may have optimal efficacy when used in the setting of minimal residual disease (2). Furthermore, there is a good rationale for the use of combinations of antibodies or indeed for their use with traditional chemotherapy, so as to deliver a range of potentially synergistic antitumor activities (4–6).

The murine monoclonal antibody 30.6 recognizes an antigen that is expressed on colorectal carcinomas and their metastases. Expression of the antigen is greatest on well-differentiated colorectal adenocarcinomas, is less pronounced on poorly differentiated adenocarcinomas, and is usually absent from most undifferentiated carcinomas (7). The biochemical nature of the 30.6 antigen has not been elucidated, but it is expressed only on the luminal surface of glandular cells and is not released into the circulation. The antigen is found in gastrointestinal epithelium as well as in pancreatic acini, hepatocytes, alveolar pneumocytes, and prostatic acinar epithelium. However, it is not expressed in other organs or tissues of the urogenital tract or central nervous system (7). The murine 30.6 antibody has been shown to localize to s.c. human colorectal cancer xenografts in nude mice (8) as well as to primary and secondary tumor deposits in patients with metastatic colorectal cancer (8, 9).
Furthermore, the antibody, whether used alone, radiolabeled, or conjugated to cytotoxic drugs, is able to strongly inhibit the growth of human colorectal carcinoma xenografts in nude mice (10). Phase I clinical studies of N-acetyllymphophan coupled to 30.6 showed that patients produced a HAMA\(^5\) that precluded additional dosage escalation (11, 12).

The development of HAMA could impair the therapeutic effectiveness of 30.6, either by interfering with its binding to antigen or by reducing its bioavailability. Replacing the murine constant regions with a human \(\gamma_1\) region was likely to reduce the immunogenicity of the antibody and also maximize its effector function. Mount et al. described a chimeric version of this antibody (c30.6) that binds with moderate affinity (\(K_a = 1 \times 10^8\) \(\text{M}^{-1}\)) to its antigen and can mediate in vitro ADCC (13). Although this chimeric antibody was not able to lyse cells in the presence of either human or rabbit complement, it had antitumor activity in mice with SCID bearing s.c. human colorectal cancer xenografts. A 40% reduction in tumor size was observed after i.p. c30.6 administration, with maximal antitumor activity while the c30.6 antibody was being administered.

The chimerized 30.6 antibody, therefore, has a number of characteristics that make it an attractive antibody for additional clinical development. These include its ability to induce ADCC, its pattern of tissue reactivity, affinity, potential for reduced immunogenicity, and documented antitumor activity in animal models. We report here the results of single-dose escalation studies of the chimeric 30.6 monoclonal antibody in patients with metastatic colorectal cancer. The primary objectives of the study were to evaluate safety, pharmacokinetics, and biodistribution of the c30.6 antibody in this patient population.

**PATIENTS AND METHODS**

**Production of c30.6.** Recombinant c30.6 (IgG1κ) antibody is secreted from CHO cells. The cloned 30.6 antibody heavy- and light-chain, variable region cDNAs (obtained from the Austin Research Institute, Melbourne) were subcloned into the antibody expression vectors pG1D102 and pKN100 (Medical Research Council, Cambridge, United Kingdom). The vectors containing heavy and light chains were transfected into the host cell line CHO DG44 (from Dr. Larry Chasin, Columbia University, New York). After selection and screening, the production cell line 10A75H2.2F5 was isolated. The master cell bank of 10A75H2.2F5 was found to be free of microbial contaminants, murine adventitious virus, and retrovirus. As expected, transmission electron microscopy demonstrated the presence of endogenous retrovirus particles in the CHO cells. c30.6 antibody was manufactured using batch fermentation with serum-free media and was purified via a multistep procedure incorporating five chromatography and two viral inactivation/removal steps. The antibody was separated by Protein A affinity chromatography and then treated with solvent/detergent to inactivate lipid-enveloped viruses. Additional purification by anion and cation exchange chromatography removed residual proteins, solvent/detergents, and nucleic acids. The purified c30.6 was additionally purified and formulated into 0.9% saline using two gel filtration columns. Finally, the formulated bulk was sterile- and viral-filtered before dispensing into Hypak glass syringes (Becton Dickinson, Lincoln Park, NJ). Stability studies showed that the c30.6 antibody was stable in this formulation for at least 16 months. Purity, identity, activity, and safety were confirmed before release of the vial product for clinical use.

**Radiolabeling of c30.6.** The c30.6 antibody (5 mg in 2 ml) was labeled with \(^{123}\text{I}\) (2 GBq in 1 ml; ARI, Sydney) using the Iodogen method, and the labeled antibody was purified by gel filtration using a Superose 12 HR column (Pharmacia, Uppsala, Sweden) coupled with fast protein liquid chromatography. Before purification, the iodine incorporation was 68.0 ± 7.0%. The immunoreactive fraction of the labeled antibody was estimated by the Lindmo method (14) using the human colorectal cell line HT-29. The average immunoreactivity of the radio-labeled preparations was 60.7 ± 8.6%. The radiolabeling method was validated to produce sterile and pyrogen-free product. Serum samples from patients injected with radiolabeled antibody were analyzed by gel filtration chromatography for the presence of aggregates and free iodide using a Superose 12 HR10/30 gel filtration column.

**Patient Selection.** For entry to the study, the patients were required to have histologically proven metastatic colorectal cancer and to have adequate renal (creatinine <125% of the upper limit of the normal range), hepatic (bilirubin <125% of the upper limit of the normal range; prothrombin time < 1.3 times control), and hematological function (white blood count >4.0 \(\times 10^9\)/liter; platelet count >100 \(\times 10^9\)/liter; hemoglobin >100 g/liter) as well as the presence of measurable metastatic disease (at least one site ≥ 1 cm). Each patient was also required to have a WHO performance status of ≤ 2 and a life expectancy of at least 3 months. Patients were excluded if they had undergone chemotherapy or radiotherapy in the preceding 4 weeks or had received immunosuppressive therapy in the preceding 3 months.

The 30.6 antigen is not detectable in paraffin embedded tissues, but is expressed on almost all of the moderately and most of the poorly differentiated adenocarcinomas of the colorectum (7), and therefore documentation of antigen expression was not required for entry into the study. Patients with severe nonmalignant systemic disease or who were HIV positive or had uncontrolled infection were precluded from entry. Those individuals who had previously been exposed to murine or chimeric antibody or antibody fragments were also excluded from entry. Detailed informed consent was obtained from all of the patients in accordance with the St. Vincent’s Hospital Human Ethics Committee.

**Clinical Trial Design.** The first four patients entered in this study received 3 mg \(^{123}\text{I}\)-labeled c30.6 (30 mCi). The next 13 patients received a single dose of antibody at doses of 10 (5 patients), 25 (5 patients), and 50 mg/m\(^2\) (3 patients). Immediately after the infusion of unlabeled antibody, 6 of the 13 patients also received 3 mg \(^{123}\text{I}\)-labeled c30.6 antibody. Patients were selected for administration of \(^{123}\text{I}\)-labeled c30.6 on the

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\(^5\) The abbreviations used are: HAMA, human antimouse antibody response; ADCC, antibody-dependent, cellular cytotoxicity; SCID, severe combined immunodeficiency; CHO, Chinese hamster ovary; HACA, human anti-c30.6 antibody; CEA, carcinoembryonic antigen; CT, computed tomography; PET, positron emission tomography; SPECT, single-photon emission computed tomography; H1, histamine receptor 1.
basis of the site and extent of their disease as well as performance status. Those individuals who received radiolabeled antibody were treated with Lugol's iodine for 3 days before and 2 days after the antibody infusion.

**Study Measurements.** All of the adverse events that occurred within 28 days of administration of the antibody were recorded and graded according to the Southwest Oncology Group Criteria. Measurement of hematology, serum chemistry, liver function tests, complement, HACA, and serum c30.6 levels were performed at regular intervals for a period of 6 months after the treatment. Physical examination, serum CEA levels, CT and PET imaging studies were performed at 4 to 6 weeks after treatment to assess the tumor response. A partial response was defined as a decrease of >50% in the total sum of the products of the bidimensional measurements. Complete remission was defined as the disappearance of all of the disease, whereas stable disease was defined as no significant change in tumor measurements. A single expert (G. B.-W.) who was unaware of the treatment protocol examined all of the imaging studies.

**Pharmacokinetics of c30.6.** To determine the pharmacokinetics of c30.6, serial blood samples were assayed by either gamma counting to detect radiolabeled c30.6 (used in the first four study patients) or ELISA to detect both unlabeled and radiolabeled antibody (used in all other study patients). In each patient who received radiolabeled antibody, EDT- treated blood samples were collected at 2, 4, 8, 15, and 30 min and 1, 4, 24, and 48 h. After 48 h 1-ml plasma samples were counted on a gamma counter (autogamma 5650; Packard Instruments, Downers Grove, IL). The total administered dose was determined using a dose calibrator, and the disintegrations per minute were calculated by correcting for the efficiency of the gamma counting.

Serum c30.6 levels were determined using a competitive, solid-phase enzyme immunoassay, in which chimeric antibody in serum competes with exogenous biotin-labeled c30.6 for binding to sheep anti-c30.6 antibodies coated onto ELISA plates. Plates (Nunc-Immuno MaxiSorp) were incubated overnight at 4°C with affinity-purified sheep anti-c30.6 antibody diluted 1:200 in coating buffer (100 mM sodium bicarbonate, 0.1% BSA, pH 8.5). After two washes in PBS/1% Tween/0.1% Brionodox, the plate was blocked with PBS/1% skim milk/0.5% BSA/0.1% Bronidox for 90 min at 37°C.

Patient serum samples were collected before the infusion, at 1, 4, 24, and 48 h, and again at 6, 8, and 15 days. Samples and standards were heat-inactivated (60°C, 30 min), diluted in pooled heat-inactivated human serum, and then mixed with an equal volume of biotinylated c30.6 (1:2000 in blocking buffer diluted in PBS/0.1% skim milk/0.05% BSA/0.1% Bronidox). This mixture was incubated on the washed plate at 37°C for 2 h, washed, and then incubated with alkaline phosphatase-streptavidin (1:2000 in sample diluent; Jackson ImmunoResearch Laboratories, West Grove, PA) for 60 min at 37°C. After additional washes, p-nitrophenyl phosphate in carbonate buffer was added, and the plate was incubated at 37°C until the lowest point on the standard curve (1 ng/ml c30.6) had an absorbance at 410 nm of 2.0 (MR 7000 Microplate Reader; Dynatech). Each assay included a set of standards of c30.6 (2–1000 ng/ml) diluted in pooled human serum and mixed with biotinylated c30.6. Standard curves and serum antibody concentrations were calculated using the Assay-Zap assay program (Elsevier-Biosoft, Cambridge, United Kingdom). The detection limit of the assay was 20 ng/ml, although c30.6 levels of 1 ng/ml were detectable. Pharmacokinetic data analysis was performed using the nonlinear parameter estimation application Minim (Dr. Robert Purves, University of Otago Medical School, Dunedin, New Zealand).

**Measurement of HACA Levels.** HACA levels were determined using a bridging ELISA. In this assay, serum HACAs act as a bridge between c30.6 antibody coated on an ELISA plate and biotinylated c30.6 (sulfo-NHS-LC-biotin; Pierce) in solution. A signal, therefore, indicates that biotin has been linked to the solid phase and that anti-c30.6 antibodies were in the serum sample. Plates were coated with c30.6 for 16 h at 4°C (2 μg/ml in coating buffer) and blocked as described above. After the addition of biotinylated c30.6, patient serum samples were added to the plate. Each plate also included the following controls: goat antihuman IgG Fc antibody (0.1–1000 ng/ml; Jackson ImmunoResearch) for quantitation of the HACA response, sheep anti-c30.6 as a positive control and patient baseline serum samples as negative controls. Alkaline phosphatase-streptavidin was added to washed plates, and color was developed and analyzed as described above. The limit of detection of this assay was 2 ng/ml of goat antihuman IgG Fcγ.

**Imaging and Dosimetry Studies.** In those patients who received 125I-labeled c30.6, anterior and posterior whole-body planar images (256 × 1024 matrix, 15 min/m) were performed immediately and at 1, 24, and 48 h after injection. In addition SPECT images (128 × 128 matrix, elliptical orbit, 64 views, 360°, 30 s/view) of the chest and abdomen were obtained at 24 and 48 h. A GE XRT gamma camera and high-resolution, low-energy collimator was used for all of the imaging. Radiation dosimetry was estimated by using the whole-body images from 0, 4, 24, and 48 h after injection. The cumulated activity was calculated for various organs of interest and then converted into residence times. These times were entered in to the MIRDOSO program (Oak Ridge National Laboratories) to determine the internal radiation dosimetry.

**RESULTS**

**Patient Characteristics.** The characteristics of the 17 patients with progressive metastatic colorectal cancer (7 rectal, 10 colon) who entered this study are shown in Table 1, along with a summary of prior therapies and sites of disease at the time of administration of c30.6. The participants were aged from 37 to 78 years, and the sites of their metastatic disease included the liver (n = 16), lungs (n = 5), mesentry (n = 2), lymph nodes (n = 5), bones (n = 3), adrenal (n = 1), and pelvis (n = 2). Seven individuals had initially presented with metastatic disease, and in two of these cases the primary tumor had not been resected before antibody administration. Of the 10 individuals without metastatic disease at initial presentation, 5 had received adjuvant 5-fluorouracil-based chemotherapy shortly after resection of their primary tumor, and one of these individuals developed metastatic disease while undergoing adjuvant therapy.

At the time of antibody therapy, chemotherapy was considered inappropriate in five patients, either because of progressive disease despite palliative therapy (n = 3), relapse on
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex, Age</th>
<th>Dose (mg)</th>
<th>Primary site</th>
<th>Primary resected</th>
<th>Metastases at presentation</th>
<th>Prior surgery*</th>
<th>Prior radiotherapy*</th>
<th>Prior chemotherapy*</th>
<th>Sites of disease at time of c30.6 treatment*</th>
<th>Response determined by CT/PET scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 37, M</td>
<td>2.1c</td>
<td>SC</td>
<td>Y</td>
<td>Y</td>
<td>LAR (3 wk)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Multiple bilateral hepatic left cervical lymph node, hepatic (10–40 mm), pulmonary, mesentry, right adrenal</td>
<td>NA, withdrew from study 6 lesions P, 2 NC</td>
</tr>
<tr>
<td>2 78, M</td>
<td>3.1c</td>
<td>DC</td>
<td>Y</td>
<td>N</td>
<td>LH (3 yr)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (3 yr)</td>
<td>1 right hepatic lobe (20 mm), nodes at porta hepatis and around duodenum</td>
<td>2 new lesions, 2 P, 2 NC</td>
</tr>
<tr>
<td>3 57, M</td>
<td>3.2c</td>
<td>R</td>
<td>Y</td>
<td>N</td>
<td>LAR (3 yr)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant pelvis (4 yr)</td>
<td>Bilobar hepatic (10–15 mm) and left femur</td>
<td>2 P</td>
</tr>
<tr>
<td>4 58, M</td>
<td>3.1c</td>
<td>TC</td>
<td>Y</td>
<td>Y</td>
<td>STC (6 mo)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (4 yr)</td>
<td>2 hepatic metastases (8 &amp; 60 mm)</td>
<td>2 P</td>
</tr>
<tr>
<td>5 67, M</td>
<td>17</td>
<td>R</td>
<td>Y</td>
<td>N</td>
<td>AR (4 yr)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (2 mo)</td>
<td>Right hepatic metastases (20–50 mm)</td>
<td>ND (died at 8 wk)</td>
</tr>
<tr>
<td>6 52, M</td>
<td>17, 3.9c</td>
<td>AC</td>
<td>Y</td>
<td>N</td>
<td>RH (9 mo)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (2 mo)</td>
<td>Bilobar hepatic metastases (10–20 mm), rectal tumor infiltrating prerectal space</td>
<td>3 hepatic lesions, NC</td>
</tr>
<tr>
<td>7 51, F</td>
<td>13.8, 3.0</td>
<td>R</td>
<td>N</td>
<td>Y</td>
<td>ND</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant pelvis (2 yr); palliative left iliac crest (9 mo)</td>
<td>Bony, pelvis, pulmonary</td>
<td>On CT, NC on PET, P in lung but response in pelvis</td>
</tr>
<tr>
<td>8 56, M</td>
<td>16, 3.0c</td>
<td>R</td>
<td>Y</td>
<td>N</td>
<td>AR (5 yr); AP (4 yr)</td>
<td>Palliative pelvis (2 yr); palliative local recurrence (3.5 yr)</td>
<td>Adjuvant pelvis (4 yr)</td>
<td>Palliative i.a. 5FU/Mito (18 mo); palliative i.v. 5FU/Mito/FA (6 mo); camptosar (8 wk)</td>
<td>Bony, pelvis, pulmonary</td>
<td>On CT, NC on PET, P in lung but response in pelvis</td>
</tr>
<tr>
<td>9 72, M</td>
<td>13.8</td>
<td>R</td>
<td>Y</td>
<td>N</td>
<td>AR (4.5 yr)</td>
<td>Adjuvant pelvis (4.5 yr)</td>
<td>Adjuvant 5FU/FA (4 yr)</td>
<td>Adjuvant 5FU/FA (6 mo); campotosar (8 wk)</td>
<td>Right hilum, para-aortic lymph nodes, hepatic</td>
<td>ND, refused follow up CT</td>
</tr>
<tr>
<td>10 69, M</td>
<td>40</td>
<td>R</td>
<td>Y</td>
<td>N</td>
<td>LAR (6 yr)</td>
<td>Palliative, local recurrence (3.5 yr)</td>
<td>Palliative, radiosensitizer 5FU (3.5 yr)</td>
<td>Palliative, radiosensitizer 5FU (3.5 yr)</td>
<td>Left hepatic, pelvis, pulmonary (10–40 mm)</td>
<td>1 new pulmonary lesion, 2 P</td>
</tr>
<tr>
<td>11 69, M</td>
<td>45, 3.0c</td>
<td>DC</td>
<td>N</td>
<td>Y</td>
<td>ND</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (4 yr)</td>
<td>Bilobar hepatic metastases (5–30 mm), descending colon tumor</td>
<td>Slight P hepatic lesions, response in primary tumor</td>
</tr>
<tr>
<td>12 75, M</td>
<td>47.5</td>
<td>SC</td>
<td>Y</td>
<td>N</td>
<td>PC (18 mo)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (18 mo); palliative SFU (3 mo)</td>
<td>Bilobar hepatic, Bony L2, T10, left iliac crest</td>
<td>ND (died at 18 days)</td>
</tr>
<tr>
<td>13 42, M</td>
<td>48, 3.0c</td>
<td>AC</td>
<td>Y</td>
<td>Y</td>
<td>RH (2 yr)</td>
<td>Palliative mediastinum (3 mo)</td>
<td>Palliative 5FU/FA (18 mo); palliative SFU (3 mo)</td>
<td>Palliative 5FU/FA (18 mo); palliative SFU (3 mo)</td>
<td>Hepatic (up to 80 mm), pulmonary (2 mm), paraaortic LNs (up to 20 mm)</td>
<td>4 NC (lung, liver, lymph nodes)</td>
</tr>
<tr>
<td>14 73, M</td>
<td>47.5</td>
<td>SC</td>
<td>Y</td>
<td>N</td>
<td>AR (18 mo)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (1 yr)</td>
<td>Hepatic (2–30 mm)</td>
<td>2 lesions P, 2 NC</td>
</tr>
<tr>
<td>15 56, M</td>
<td>95, 3.0c</td>
<td>AC</td>
<td>Y</td>
<td>N</td>
<td>RH (4 yr); Local wound metastases (6 mo)</td>
<td>Palliative 5FU (3 mo)</td>
<td>Palliative 5FU (3 mo)</td>
<td>Palliative 5FU (3 mo)</td>
<td>Bilobar hepatic and omental disease</td>
<td>CT scan ND; suggestion of early response on PET</td>
</tr>
<tr>
<td>16 70, M</td>
<td>95</td>
<td>HF</td>
<td>Y</td>
<td>Y</td>
<td>RH (3 mo)</td>
<td>Nil</td>
<td>Nil</td>
<td>Adjuvant 5FU/FA (1 yr)</td>
<td>Bilobar hepatic metastases (15–30 mm), portocaval node (20 mm)</td>
<td>2 hepatic lesions P, 2 hepatic lesions NC, lymph node NC</td>
</tr>
<tr>
<td>17 63, M</td>
<td>90</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>AP (4 mo)</td>
<td>Adjuvant pelvic (2 mo)</td>
<td>Radiosensitizer SFU (2 mo)</td>
<td>Radiosensitizer SFU (2 mo)</td>
<td>Bilobar hepatic metastases (5–20 mm)</td>
<td>CT scan ND, P on PET</td>
</tr>
</tbody>
</table>

*a Time since last treatment in parentheses.

*b Size range in parentheses.

c Dose in milligrams of radiolabeled c30.6 administered after unlabeled antibody.

'd SC, sigmoid colon; R, rectum; DC, descending colon; TC, transverse colon; HF, hepatic flexure; AC, ascending colon; AP, abdominoperineal resection; RH, right hemicolectomy; PC, proctocolectomy; AR, anterior resection; LAR, low anterior resection; STC, subtotal colectomy; LH, left hemicolectomy; ND, not done; 5FU, 5-fluorouracil; FA, folinic acid; i.a., intraarterial; Mito, mitomycin C; NA, not assessable; NC, no change; P, progressed.
adjuvant therapy \((n=1)\), or refusal to undertake palliative therapy \((n=1)\). An additional 12 individuals had progressive disease but were as yet asymptomatic or their symptoms were well controlled with other medications.

### Antibody Infusions

The unlabeled c30.6 antibody was administered in an outpatient clinic by infusion through a peripheral line. Dose levels of 10, 25, and 50 mg/m\(^2\) were diluted in 50, 100, or 500 ml 0.9% sodium chloride and delivered over 40, 60, or 120 min, respectively. Ten individuals were injected with a slow i.v. push of \(^{123}\)I-labeled c30.6 (30 mCi), either alone (patients 1–4) or immediately after the completion of the unlabeled antibody infusion (patients 6–8, 11, 13, 15; Table 1). The first seven patients (1–7) did not receive a premedication before the treatment, whereas the next seven (8–14) were premedicated with a combination of loratadine (5–10 mg) and paracetamol (500 mg) up to 1 h before antibody administration. The final three patients (15–17) were premedicated with promethazine (50–75 mg i.m.) 2 h before antibody infusion and with ranitidine (50 mg i.v.) 1 h before treatment.

### Antibody Toxicity

All but one patient completed the scheduled infusion of antibody. In this patient the last 2 of 48 mg of antibody were not administered because he experienced uncontrollable, severe burning erythema of his face, palms, soles, and genitalia. A total of 56 adverse events occurred in the first 24 h after infusion of antibody, and of these 55 were directly attributable to the antibody infusion (Table 2). The most frequent side effect (56% of all of the adverse events) was a constellation of symptoms consisting of severe burning and erythema of the face, chest, neck, ears, palms, soles, and genitalia (penis, testis, vagina, external labia). This reaction was often accompanied by conjunctival injection, itching of the external auditory canal, injection of nasal mucosa, stuffy nose, and discomfort or burning around the lips and throat. These symptoms typically began 30–300 min after the infusion commenced (median, 70 min) and lasted from 5 min to 4.3 h (median, 1.5 h). Although each patient did not invariably experience burning erythema at each site, the temporal involvement of the skin typically followed the following sequence: face/genitalia, palms, and then soles. These symptoms were self-limiting and did not require admission to hospital, yet in some patients they were of such severity as to require narcotic analgesia. Once these symptoms had begun, they were refractory to all of the modalities of treatment, including cold packs, drugs such as H1 blockers (promethazine, 25–50 mg i.v.), hydrocortisone (up to 200 mg iv), Sudafed (60 mg p.o.), paracetamol, and narcotics. Unfortunately, the severity of pain meant that skin biopsies of affected sites could not be performed. At the dose level of 10 mg/m\(^2\) all 5 patients experienced these mucocutaneous side effects, and of the 10 events, 2 were mild, 6 were moderate, and 2 were severe. At the 25-mg/m\(^2\) level, four of the five patients demonstrated this side effect, with four mild, five moderate, and four severe reactions.

These reactions occurred despite pretreatment in some cases with loratadine and paracetamol 1 h before infusion (2 of the 5 individuals at the 10-mg/m\(^2\) dose level and all of the individuals at the 25-mg/m\(^2\)dose level). At the 50-mg/m\(^2\) dose level of the patients at the 10-mg/m\(^2\) dose level and all of the individuals at the 25-mg/m\(^2\)dose level). At the 50-mg/m\(^2\) dose level of the patients at the 10-mg/m\(^2\) dose level and all of the individuals at the 25-mg/m\(^2\)dose level). At the 50-mg/m\(^2\) dose level, promethazine and ranitidine was given to all three patients and resulted in a dramatic improvement in the severity of this syndrome, with all of the events being reported as mild.

In an attempt to additionally elucidate the nature of this mucocutaneous reaction, tryptase was measured in serum sam-

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**Table 2**  Acute adverse events related to infusion of the c30.6 antibody

An acute event was defined as a side effect occurring within 24 h after treatment with the antibody. Those events that were either definitely or possibly a consequence of antibody administration are included in the table.

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Radiolabeled ((n=4))</th>
<th>10 ((n=5))</th>
<th>25 ((n=5))</th>
<th>50 ((n=3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rigors and/or temperature</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Shoulder/hip pain</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sneezing</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning erythema (eyes, external auditory canal, nasal mucosa)</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Burning erythema (palms, soles)</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Burning (genital)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Burning erythema (face, ears)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5</td>
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<tr>
<td>Rash</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tumor pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Right hip</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sciatica</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total adverse events</td>
<td>2</td>
<td>15</td>
<td>21</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^*\ n is patients per group. 
\(^2\ ML, mild; MD, moderate; S, severe.
Full Blood Count/Biochemistry/Liver Function Tests, and Complement. No clinically significant changes in hematology, biochemistry, or complement (C3, C4, CH50), occurred in the 4-week period after antibody administration. There was considerable interpatient variability in the liver function tests measured in the first 28 days. For instance, the alkaline phosphatase fell by 50% in some individuals, whereas in others it increased to 30% above baseline. Similar changes were noted in γ-glutamyltransferase and the transaminases. Overall there was no significant change in liver function tests, which depended on patient dose or predicted response or was discordant with the pattern of their liver function tests in the subsequent 3 months.

Pharmacokinetics. Pharmacokinetic analysis of plasma $^{123}$I-labeled c30.6 levels showed a biexponential clearance pattern with an α half-life of 2.5 ± 0.7 min (mean ± SD) and a β half-life of 46 ± 15 h. An ELISA was used to determine the serum c30.6 concentrations in those patients treated at the 10-, 25-, and 50-mg/m² dose level (Fig. 1). At the 10 mg/m² dose the β half-life was 51 ± 5 h, and this increased to 57 ± 19 h at 25-mg/m² dose level and 81 ± 15 h at 50 mg/m². The maximum observed serum concentration increased from 0.97 μg/ml at the 10-mg/m² level to 2.4 μg/ml at 25-mg/m² and 7.9 μg/ml at 50-mg/m² levels. Importantly, at the 50-mg/m² dose level, the serum concentration of c30.6 remained at ~100 ng/ml from 1 week to at least 3 weeks after the infusion. It is also of note that there was marked interpatient variability in all of the pharmacokinetic parameters.

HACAs. Serum samples were assayed for HACAs at 2 and 6 weeks as well as at 3 and 6 months. One of 17 patients manifested a HACA response, and this was detectable at 14 days to 6 months posttreatment. This patient received only 2.1 mg $^{123}$I-labeled c30.6 and was not treated with any unlabeled antibody. The level of HACAs was low (14 ng/ml at 3 and 6 months) and could be detected only in neat serum. In comparison, sheep immunized with c30.6 have anti-c30.6 levels of ~400 μg/ml in this assay.

Tumor Response. Whereas all 17 patients had measurable disease, tumor response was only assessable in 13 of these individuals (Table 1). Two patients died before imaging could be undertaken (one from a myocardial infarct and the second from progressive disease) and an additional two patients refused follow up scans (one withdrew from study and the other remained on study but did not undergo imaging). There were no
partial or complete responses in the 10 assessable patients who received unlabeled c30.6, with or without $^{123}$I-labeled c30.6. At 4–6 weeks, five patients had progressive disease, and two had stable disease. The remaining three patients (8, 13, 15) had evidence of improvement in at least some of their metastatic lesions. For instance, in patient 8 there was no change in the CT scan performed at 7 weeks; however, the PET scan demonstrated a 22–30% reduction in glucose metabolism at measurable sites within the extensive pelvic disease. The PET scan also showed progressive disease in the chest, with an increase in the size and avidity of lesions at the base of right lung, hilum, and mediastinum. This progression was not noted on CT but subsequently became readily apparent. Figure 2 shows the results of PET scanning of patient 13, where the qualitative decrease in glucose avidity of some hepatic lesions is readily apparent at 7 days, with an additional decrease at 6 weeks. The 6-week scan also identified new lesions, particularly in the chest. These new lesions were also not detected on CT. Patient 15 refused CT scanning, but a PET scan performed at day 13 demonstrated a 7% decrease in glucose uptake in the extensive intra-abdominal disease. By 7 weeks, this patient had deteriorated clinically, and PET scanning confirmed progressive disease, with a 24–35% increase in glucose uptake.

The median percentage change in CEA from baseline to 8 weeks was found to be $+11\%$ (range, $-29$ to $+169$), $0\%$ (range, $-38$ to $+132$), and $+6\%$ (range, $0$ to $+54\%$) at the 10-, 25-, and 50-mg/m$^2$ dose levels. All patients in whom PET scanning showed some reduction in particular sites of disease were also found to have small increases in serum CEA levels, probably reflecting the previously mentioned occurrence of progression at other sites. Other patients, such as patient 10, demonstrated a sustained decrease in CEA from 195 $\mu$g/liter at baseline to 120 $\mu$g/liter at 8 weeks (normal <3.0 $\mu$g/liter), yet CT scanning clearly showed progression of disease.

**Biodistribution and Imaging.** Gel filtration chromatography was used to analyze serum collected at 4, 24, and 48 h from two patients who received only $^{123}$I-labeled c30.6. The radioactivity from all of the samples eluted as a single peak at a $M_r$ of 160,000, indicating that there was no detectable complexing or breakdown of the labeled antibody *in vivo*. Further evidence supporting the *in vivo* stability of the antibody and label was provided by analysis of 48-h urine collections, which showed that 17–21% of the injected activity was excreted in this period.

Whole body planar images obtained at 0, 4, 24, and 48 h after injection indicated that there was a very rapid and intense uptake of activity in the liver, coupled with a decrease in the blood pool activity over the first hours (Fig. 3). At the zero time point, planar images of the four patients injected with $^{123}$I-labeled c30.6 (patient 1–4; Table 1) showed that 50% (range, 43–54%) of the dose localized in the liver, and at 48 h the localization remained high at 44% (range, 35–50%). However, as expected, the preinfusion of unlabeled c30.6 significantly reduced the hepatic uptake of $^{123}$I-labeled c30.6 (mean hepatic localization, 25%; range, 21–35% at the zero time point). In patients 1–4 the mean whole-body effective dose equivalent was $3.19 \pm 0.37 \times 10^{-2}$ mGy/MBq.

SPECT obtained at both 24 and 48 h after $^{123}$I-labeled c30.6 injection identified primary and some metastatic lesions. The two primary tumors (patients 7 and 11) that had not been resected before study were successfully imaged as hot lesions with radioactivities of 1.2 and 2% (Fig. 4A). Hepatic lesions were visualized either as hot lesions, cold lesions, or regions of low activity surrounded by a hot rim (Fig. 4B). Although all of

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**Fig. 2.** PET fluorine-18-deoxyglucose (FDG) scanning of patient 13 after injection of 48 mg c30.6 antibody. Scans taken at baseline (A) and again at 7 days (B) and 6 weeks (C). The percentages of reduction in glucose uptake in (B) and (C) were 12–14% and 24–28%, respectively, compared with baseline.
the individuals with hepatic disease were reported to have abnormal areas of hepatic uptake, SPECT did not identify all of the lesions reported on CT and PET scanning. As expected, the pattern of uptake was somewhat dependent on the size of the lesion. This observation is consistent with previous reports in which lesions with a diameter of >2.5 cm usually appeared as cold defects with or without a hot rim, whereas lesions ≤2.0 cm tended to show high uptake of radioactivity (15). Although two patients with extra-abdominal disease (patients 2 and 13) participated in the study, SPECT imaging did not identify the pulmonary and nodal tumor sites in either case. However, one individual with extensive pelvic disease did demonstrate a quite marked uptake of isotope at these sites (patient 8).

**DISCUSSION**

In this study we describe the generation of a novel chimeric antibody and its clinical evaluation in individuals with metastatic colorectal cancer. To obtain additional data on biodistribution, pharmacokinetics, and tumor targeting, we also treated a subset of patients with radiolabeled c30.6 antibody. I was chosen for this purpose, because its short half-life (13 h) and low-energy γ emission (159 keV) allow for safe handling and improved imaging.

This study demonstrated that I-labeled c30.6 antibody was of insufficient specificity or sensitivity for use as a diagnostic imaging agent. Although some lesions such as primary colorectal tumors were readily detected, other large tumor deposits in the lungs and lymph nodes remained occult. The detection of hepatic lesions was clearly disadvantaged by the combination of the high uptake of antibody in the liver and the short half-life of the label. Previous studies have demonstrated that this type of imaging problem can be overcome by the use of antibody fragments and alternative labels (16–18). The whole-body planar images graphically demonstrated the intensive and prolonged uptake of antibody into the normal liver parenchyma. Furthermore, it was shown that this nonspecific uptake could be reduced by the preinfusion of cold antibody. It is likely that nonspecific hepatic uptake was one of the factors determining the antibody half-life and maximum serum concentrations. These parameters increased as the dose of infused antibody was escalated, probably as a result of saturation of hepatic binding sites. The reservoir of antibody in the liver and its slow release back into the circulation may also account for the prolonged maintenance of serum c30.6 levels in patients who received doses of 50 mg/m². Despite the intensive hepatic uptake, there was no biochemical or clinical evidence of hepatotoxicity.

The serum half-life and maximum serum concentration seen with c30.6 (81 h, 7.9 μg/ml at the 50-mg/m² dose) is comparable with that of other chimeric antibodies presently in clinical trial. For instance the half-life of chimeric 17.1A was 100 h, and the maximum serum concentration was 11 μg/ml when used at a doses of 40 mg fortnightly for three infusions (19). Similarly, the IDEC-C2B8 had a half-life of 33 h after a dose of 375 mg/m², and this increased to 76 h after four infusions, as a result of saturation of CD20 sites in the serum and circulation (1). On the basis of these and other studies, we anticipate that the half-life and maximum serum concentration of c30.6 will increase after the infusion of multiple doses.

Most side effects occurred within 4 h of the antibody
infusion, were self-limiting, and did not require admission to hospital. Some of these side effects such as rashes and headache have been frequently observed with other antibody therapies (3, 20, 21). The gastrointestinal side effects and in particular the episodes of nausea and vomiting were also predictable, because c30.6 extensively cross-reacts with the gastric mucosa.

One patient who received the highest dose of c30.6 developed an antibody-induced pancreatitis, the symptoms of which were mild and self-limiting and were disproportionate to the degree of elevation of serum amylase that was observed. It is probable that the pancreatitis resulted from binding of antibody to pancreatic structures. This may have taken the form of direct cytolysis of acinar cells or obstruction secondary to cross-reactivity with ductal epithelium. Cross-reactivity against structures in the normal pancreas had previously been identified in immunohistochemical studies on postmortem tissues; however, precise localization of the 30.6 antigen was not possible because of the presence of extensive autolysis, as is typical of this tissue. Although the serum amylase was normal in six other individuals, it seems that infusion of antibodies, e.g., other drugs such as thiazide diuretics and azathioprine, can cause pancreatic injury (22).

To our knowledge, the constellation of mucocutaneous symptoms seen in this study has not been described with other monoclonal antibodies or indeed with any other form of drug therapy. The temporal progression of burning cutaneous erythema involving at first the face and then the chest, genitalia, palms, and soles, in succession, was particularly distinctive. Pain at these sites was often of such severity that it necessitated the use of narcotics. Although there was some variability between individuals, it was apparent that the frequency and severity of this reaction increased proportionally to the dose of antibody administered.

Interestingly, this reaction occurred in the absence of symptoms classically associated with hypersensitivity reactions, such as bronchospasm, urticaria, facial and laryngeal edema, and hypotension. However, although the established reaction was refractory to all attempted therapies, it was readily prevented by premedication with high doses of combination H1 and H2 antagonists. The importance of high doses of these antagonists was recently illustrated in a follow up multidose trial of c30.6, where a 25% reduction in the dose of the H1 antagonist was associated with recurrence of this syndrome (unpublished observations).

A number of pathogenic mechanisms can be considered in regard to this mucocutaneous reaction syndrome. The possibility that the reaction was the result of infusion of histamine contaminating the antibody preparations was excluded by retrospective analysis of all of the batches of antibody (23). A more likely explanation was that the syndrome was the result of mast cell degranulation, either directly or indirectly mediated by antibody infusion. Mast cell degranulation causes the release of a range of vasoactive and neuroactive mediators, such as histamine, tryptase, and prostaglandins (24), all of which have pharmacological actions consistent with the vasodilation, pain, and flushing of the skin seen after infusion. The protein tryptase is also released during mast cell degranulation and serves as a useful marker of this event because of its long serum half-life. However, serial estimations of tryptase and histamine in symptomatic individuals in this study failed to show elevation of serum levels. The possibility remains that mast cell degranulation may have occurred only at a local level. In this regard, elevated tryptase levels have been clearly demonstrated in bronchoalveolar and nasal lavage fluids from allergic individuals, despite the presence of unchanged normal serum levels (24–26).

Finally, it is possible that the reaction represents direct antibody cross-reactivity with the involved mucocutaneous tissues. The c30.6 antibody does show limited cross-reactivity with eccrine gland and duct epithelium, and its binding may have provided an indirect signal for mast cell degranulation. Whether the mechanism is a direct or indirect one, we postulate that the antibody induces a significant cutaneous mast cell degranulation, which is not associated with systemic effects.

The murine 30.6 was chimerized to reduce its immunogenicity and to enhance its cytotoxic effects. In a previous clinical trial of the murine antibody, all of the patients developed human anti-mouse antibodies (12), whereas only a single patient in the current study developed a HACA response. Importantly, this response was of a low level and in fact occurred in a patient who received only a low level of antibody. The lack of immunogenicity of c30.6 has allowed a multidose study to proceed, and early results indicate that no HACAs occurs after the administration of four doses.6

The c30.6 antibody may kill tumor cells in vivo by at least two possible mechanisms. In vitro data indicate that it is capable of lysing human colorectal cells in the presence of human mononuclear cells, by ADCC, although it does not lyse the same cells in the presence of either rabbit or human complement (13). Although the function the 30.6 antigen has not yet been defined, it is also possible that the binding of antibody could exert a direct cellular effect, in a manner similar to the effects of antibodies, which bind to the HER-2/neu receptor (27, 28). Despite these potential actions, there were no partial responses observed in this clinical trial. This finding is not dissimilar to that of many other early studies of single doses of monoclonal antibodies in the treatment of solid tumors (29–31).

At present, antibody-based therapeutics represent an expensive treatment option. In initial phase studies of new antibodies, it is therefore important to identify those modes and time points for imaging studies that will allow the earliest detection of treatment responses. In this regard, an important observation from this study was the potential role of PET scanning. Using this modality, we found that three patients had some objective disease improvement, an event that was paralleled by symptomatic improvement in two of those cases. This suggests that PET scanning may prove more sensitive than computerized tomography in the early detection of treatment response, particularly in the setting of extensive or bulky disease. A current limitation of PET scanning is the difficulty in precisely quantifying responses. Nevertheless, it is likely that this modality, perhaps when used in conjunction with other imaging tools, will provide a useful means for the early identification of responses to monoclonal antibody therapy. We are presently investigating this issue as part of a multidose study with 30.6.

In summary, the chimerized c30.6 antibody is not immunogenic in humans and appears worthy of further study. It does, however, produce a unique profile of side effects, which although significant can be well controlled with appropriate premedication. Undoubtedly, the outcome of the multidose trial and

6 Unpublished observations.
further studies on the antigen will provide insight into its in vivo effects in the treatment of colorectal carcinoma.

REFERENCES


Phase I Clinical Trial of the Chimeric Monoclonal Antibody (c30.6) in Patients with Metastatic Colorectal Cancer

Robyn L. Ward, Deborah Packham, Anne M. Smythe, et al.


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