Phase I Clinical Comparative Study of Monoclonal Antibody KS1/4 and KS1/4-Methotrexate Immun conjugate in Patients with Non-Small Cell Lung Carcinoma


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ABSTRACT

A Phase I clinical trial was undertaken to evaluate and compare murine monoclonal antibody KS1/4 and KS1/4-methotrexate immun conjugate in patients with Stage IIIB or IV non-small cell carcinoma of the lung. Six patients received KS1/4 alone and five patients received KS1/4-methotrexate conjugate. The maximal total dose received per patient in both groups was 1661 mg. Mild to moderate side effects in both groups included fever, chills, anorexia, nausea, vomiting, diarrhea, anemia, and brief transaminasemia. One patient who received antibody alone had an apparent acute immune complex-mediated reaction. Ten of 11 patients had a human anti-mouse response. Posttreatment carcinoma biopsies revealed binding of monoclonal antibody KS1/4 and deposition of C3d and C4c complement fragments. Monoclonal antibody binding and complement deposition correlated with increasing doses of infused antibody. There was one possible clinical response.

INTRODUCTION

Murine MoAb6 KS1/4 is an IgG2a antibody that was produced to the lung adenocarcinoma cell line UCLA-P3. This MoAb recognizes a M, 40,000 and a M, 42,000 antigen found on a variety of neoplastic tissues (1). Immunoperoxidase staining of fresh frozen carcinoma suggests that the antigen is expressed by most if not all adenocarcinomas and some squamous cell carcinomas of the lung. The antigen is expressed on a normal group of epithelial cell types, suggesting that it represents an epithelial cell-derived carcinoma marker (2). Recent isolation and characterization of a cDNA encoding for the antigen have been accomplished (3). These data suggest that the antigen is a single M, 40,000 polypeptide that has heterogeneity in glycosylation, is susceptible to specific proteases, and contains a cysteine-rich domain. Sequence analysis of the 5’ and 3’ noncoding regions of the KS1/4 cDNA revealed homologies to known protooncogenes and inflammatory mediators.

MoAb KS1/4 has been conjugated to methotrexate and shown to inhibit the growth of adenocarcinoma of the lung cells, UCLA-P3, in vitro and to effectively suppress the growth of established human lung adenocarcinoma xenografts in athymic mice (4). These results suggested that the antigen that is recognized by MoAb KS1/4 may be a target antigen for antibody-directed therapy of lung carcinomas. A clinical study was, therefore, undertaken to evaluate the safety and toxicity of MoAb alone and MoAb KS1/4-methotrexate conjugate administered to patients with non-small cell carcinoma of the lung.

MATERIALS AND METHODS

Preparation of KS 1/4 and KS1/4-Methotrexate Immuno conjugate

A total of 12.5 g of unconjugated KS1/4 was prepared by Damon Biotech, Inc. (Needham Heights, MA) using an in vitro encapsulation technique. KS1/4 was covalently conjugated to methotrexate (4). Briefly, methotrexate (Lederle, Pearl River, NY) at 10 mg/ml in normal saline, pH 6.8, was mixed with EDAC at a molar ratio of 1:4 (methotrexate:EDAC) and allowed to react for 15 min at RT. Monoclonal antibody KS1/4 was added and the reaction was continued for 1 h. The KS1/4:EDAC:methotrexate molar ratios were 1:200:50 in a final reaction volume of 250 ml/g KS1/4. The conjugate was dialyzed (10 mm sodium phosphate-150 mM NaCl) and final purification was achieved on a hydroxyapatite column (Bio-Gel) using MAPS-100 high performance liquid chromatography (Bio-Rad, Richmond, CA). KS1/4-methotrexate samples (0.5 g) were applied to the column and washed with 10 mm phosphate buffer at pH 7.4 to remove free methotrexate. KS1/4-methotrexate was eluted with a linear gradient of sodium phosphate from 10 mm to 500 mm at a flow rate of 5 ml/min. The immunonconjugate eluted at approximately 300 mm sodium phosphate. Fractions containing immunonconjugate were pooled, dialyzed against normal saline, and concentrated by filtration through a YM30 Amicon filter. As measured spectrophotometrically at 410 nm, the conjugation ratio was 6 mol of methotrexate/mol of antibody (17 mg of methotrexate/g of KS1/4). Retention of antigenic specificity of the conjugate was confirmed by immunoperoxidase staining of a panel of normal and tumor tissues. Testing was performed for general safety, sterility, pyrogens, viruses, and murine DNA. The stability of the methotrexate conjugation over time was assessed by repeated dialysis of the immunonconjugate to remove free methotrexate, and subsequent spectrophotometric loss of absorbance at 410 nm was determined. Antigenic stability of KS1/4 and KS1/4-methotrexate was tested by ELISA or the ability to bind to UCLA-P3 cells following storage at −20°C for 6 months. There was no loss of binding activity at 6 months.

Patients

Eleven patients with Stage IIIB or IV non-small cell carcinoma of the lung who had failed conventional therapies were selected to receive KS1/4 or immunonconjugate. All patients were males between the ages of 41 and 70 years. Eligibility criteria required that each patient’s carcinoma express KS1/4-reactive antigen by immunoperoxidase staining. All patients had measurable or evaluable disease, a performance status of 0-2 on the Eastern Cooperative Oncology Group scale, carcinoma accessible for repeat biopsy, no chemotherapy or radiation therapy for at least 30 days prior to entry into the trial, and adequately...
preserved hematological (hemoglobin >10 g/dl, WBC >3000/mm³, platelet count >100,000/mm³), renal (creatinine <2.0 mg/dl), and hepatic (bilirubin <2.5 mg/dl, serum glutamic-oxaloacetic transaminase <70 units, alkaline phosphatase <300 units) parameters. Informed consent was obtained based on protocols on file with the Human Subjects Committee of Scripps Clinic and Research Foundation.

Study Plan

Six patients were treated with KS1/4 alone and five were treated with KS1/4-methotrexate immunoconjugate. Patients received KS1/4 or immunoconjugate in escalating doses of 1, 10, 50, 100, 500, and 1000 mg via a 4-h i.v. infusion in 100 ml normal saline with 5% human serum albumin. The infusions were given biweekly for 3 consecutive weeks for a total dose of 1661 mg of protein. The total dose of methotrexate received with the immunoconjugate preparation was 28 mg. During the course of treatment, biopsies of the tumor were obtained 24 h after a dose of KS1/4 or immunoconjugate and examined for evidence of in vivo binding of antibody, unbound KS1/4 antigen sites, and deposition of complement. Colonic mucosal biopsies were examined for the presence of KS1/4 antigen and KS1/4 antibody binding after the 1000-mg infusion. Serial blood samples were obtained at multiple time points during and following treatment for determination of KS1/4 serum levels and human anti-mouse antibody levels. Evaluation of the status of the carcinoma was based on radiographic evaluation and physical examination and was performed at time of entry and at completion of the study.

Toxicity Monitoring

A clinical research nurse monitored patients closely for any untoward reactions. Vital signs were recorded every 15–30 min during the 4-h infusions. Serial complete blood cell counts, electrolytes, and serological tests of renal and hepatic function and urinalyses were obtained during the study. Performance status and weight were evaluated daily.

Immunoperoxidase Staining

Detection of KS1/4-reactive Antigen. A two-stage indirect immunoperoxidase technique (5) was used on paraaffin-embedded and fresh frozen tissue blocks. Briefly, paraffin sections were deparaffinized and cryostat sections were air-dried, washed in PBS, and incubated for 15 min at RT in PBS containing 10% goat serum and 1% BSA. The sections were incubated for 1 h at RT with KS1/4 (1:20 dilution), washed in PBS, and incubated for 1 h at RT with HRP conjugated to goat anti-mouse antibody (Bio-Rad). Bound antibody was visualized with DAB (0.1 mg/ml) and 0.03% H₂O₂ and sections were counterstained with 1% methylene blue. Monoclonal antibody Q128 (provided by Dr. V. Quaranta) which reacts with a monomorphic determinant of the major histocompatibility complex class I antigen that is found on all cells was used as the positive control. Antibody-containing supernatants of the mouse myeloma cell line P3x63.Ag8 or the murine hybridoma 10-2.16, which is the same isotype as KS1/4 but does not react with human tissues, were used as negative controls.

Detection of Bound KS1/4 Antibody. Direct immunoperoxidase technique was used to detect KS1/4 antibody which had bound to tumor in vivo. Cryostat sections were air-dried, washed with PBS, and incubated in PBS (10% goat serum-1% BSA). Sections were covered for 1 h at RT with HRP-conjugated goat anti-mouse antibody. Negative controls were stained with HRP-conjugated goat anti-rabbit antibody and positive controls with Q128 or W6/32 as described above. Bound antibody was visualized with DAB (0.1 mg/ml) and 0.03% H₂O₂ and sections were counterstained with 1% methylene blue.

Complement Deposition at the Carcinoma Site. Murine monoclonal anti-human complement components (IgG1x isotype) anti-C3d and anti-C4c (Cytotech, San Diego, CA) were used to label bound complement. Air-dried sections were rinsed in PBS and incubated for 1 h at RT in rabbit anti-mouse antibody (Accurate, Westbury, NY) in PBS (10% goat serum-1% BSA). The rabbit anti-mouse antibody was added to block the murine MoAb KS1/4 which was already bound to the tissue sections. Sections were washed in PBS and incubated for 1 h at RT with anti-C3d and anti-C4c, in PBS (10% goat serum-1% BSA). Sections were covered for 1 h at RT with HRP-conjugated goat anti-mouse antibody. Bound antibody was visualized with DAB (0.1 mg/ml) and 0.03% H₂O₂ and sections were counterstained with 1% methylene blue. Anti-complement antibodies were omitted for the negative controls.

Serum Levels of KS1/4

Monoclonal antibody KS1/4 serum levels were measured by ELISA. Aliquots (10, 25, and 50 µl) of serum were incubated for 1 h in 96-well plates which had been coated with 5 µg/well of goat anti-mouse antibody. The plates were washed with buffer (0.02 M Tris, pH 8, containing 0.2% Tween 20) and incubated for 0.5 h with HRP-conjugated goat anti-mouse antibody. Bound antibody was quantitatively measured by reaction with o-phenylenediamine and measurement of absorbance at 490 nm in an ELISA reader (BioTek, Burlington, VT). The concentration of KS1/4 was determined by comparison with a standard curve.

RESULTS

Clinical Response. Clinical features, total administered doses of KS1/4 or KS1/4-methotrexate immunoconjugate, human anti-mouse response, maximum toxicity and clinical response are summarized in Table 1. All patients had Stage IIIB or IV non-small cell carcinoma of the lung and had received and failed conventional therapies. Each patient received a total dose of 1661 mg except for patient AS-6 who developed Grade 3 toxicity attributed to a probable acute immune complex-mediated reaction. Based on comparison of pre- and posttreatment radiographic evaluations and physical examinations, there was no decrease in measurable or evaluable disease in either group of patients. However, one patient, EH-7, may have had a clinical response. This patient had Stage IIIB disease and before entry into the study had received 6210 cGy to the lung and mediastinum. Four weeks after completion of the radiation, an endobronchial abnormality was visualized at bronchoscopy, and directed endobronchial biopsy showed histological evidence of carcinoma. After the KS1/4-methotrexate treatment, the endobronchial abnormality appeared to be smaller, directed endobronchial biopsy showed necrosis, and no viable carcinoma could be identified. This patient is alive and free of disease 43 months after immunoconjugate treatment.

Toxicity. Toxicities and side effects are summarized in Table 2. Some degree of toxicity was seen in five of six patients who received KS1/4 alone and in four of five patients who received KS1/4-methotrexate immunoconjugate. Fever and chills were seen in both groups. One of six patients in the KS1/4 group and four of five patients in the KS1/4-methotrexate group experienced mild to moderate fever and chills. Anorexia, nausea, vomiting, diarrhea, as well as mild anemia (hemoglobin 9.5–10.9), and brief increases in liver transaminases were reported in both groups. Allergic reactions such as pruritus, urticaria, and anaphylaxis were not seen. The most significant toxicity encountered was a probable acute immune complex-mediated reaction in one patient who received antibody alone. Forty-eight h after this patient had received a total dose of 661 mg of KS1/4, in the face of high human anti-mouse levels (1.8
Table 1 Summary of the clinical features, total doses of KS1/4 or KS1/4-methotrexate immunoconjugate, human anti-mouse response and maximum toxicity

<table>
<thead>
<tr>
<th>Patient entry no.</th>
<th>Histological cell type</th>
<th>Age (yr)</th>
<th>Previous treatment</th>
<th>Total dose (mg)</th>
<th>Human anti-mouse antibodies</th>
<th>Maximum toxicity</th>
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<tbody>
<tr>
<td>KS1/4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KW-1</td>
<td>Adenocarcinoma</td>
<td>66</td>
<td>Surgery, XRT, Chemo</td>
<td>1661</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td>PM-2</td>
<td>Adenocarcinoma</td>
<td>67</td>
<td>XRT</td>
<td>1661</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>CC-3</td>
<td>Large cell</td>
<td>67</td>
<td>XRT</td>
<td>1661</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>LG-4</td>
<td>Adenocarcinoma</td>
<td>67</td>
<td>Chemo, XRT</td>
<td>1661</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>RO-5</td>
<td>Squamous</td>
<td>59</td>
<td>XRT</td>
<td>1661</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>AS-6</td>
<td>Adenocarcinoma</td>
<td>43</td>
<td>Surgery, Chemo</td>
<td>661</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>KS1/4-mtx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH-7</td>
<td>Large cell</td>
<td>70</td>
<td>XRT</td>
<td>1661</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>LK-8</td>
<td>Adenocarcinoma</td>
<td>54</td>
<td>Chemo</td>
<td>1661</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>BB-9</td>
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<tr>
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<td>Squamous</td>
<td>64</td>
<td>Surgery, XRT</td>
<td>1661</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>KP-11</td>
<td>Large cell</td>
<td>53</td>
<td>XRT</td>
<td>1661</td>
<td>+</td>
<td>1</td>
</tr>
</tbody>
</table>

* XRT, radiation therapy; KS1/4-mtx, KS1/4-methotrexate; Chemo, chemotherapy.

Table 2 Summary of toxicities and side effects

<table>
<thead>
<tr>
<th>Toxicities</th>
<th>KS1/4 (no. of patients of 6)</th>
<th>KS1/4-methotrexate (no. of patients of 5)</th>
<th>Total (no. of patients of 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Rigor/chills</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Anemia</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Transaminaseemia</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Urticaria</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchospasm</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypotension</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum sickness</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

mg/ml), he developed fever to 39°C followed by muscle aches and an acute polyarthritis and synovitis involving the small joints of both hands, both elbows, shoulders, and knees. No infectious etiology was identified, erythrocyte sedimentation rate was 115, creatine phosphokinase was normal, and Raji cell assay for circulating immune complex was elevated at 190 (reference range <50 μg aggregated human gamma globulin). The patient's fever and arthritis resolved with discontinuance of MoAb infusions and administration of aspirin.

In Vivo Localization of MoAb KS1/4 and Complement Fragments. Pretreatment immunoperoxidase staining of each patient's carcinoma demonstrated expression of the KS1/4-reactive antigen. Posttreatment carcinoma biopsies obtained within 24 h after a dose of KS1/4 or immunoconjugate were examined for evidence of in vivo binding of antibody, unbound KS1/4 antigen sites, and deposition of complement. The binding of KS1/4 antibody and complement deposition in carcinoma correlated with increasing doses of antibody infused. No KS1/4 antibody could be detected in carcinoma biopsies following the 1- and 10-mg doses. Binding of KS1/4 to carcinoma as well as C3d and C4c deposition were demonstrated in some patients after the 50-mg infusion and in nearly all patients at the 500- and 1000-mg doses in both groups. There was a correlation between KS1/4 antibody binding and the deposition of complement within the carcinoma. There was no staining of carcinoma with the negative control and no complement deposition in any of the pretreatment biopsies. All posttreatment biopsies were tested for the KS1/4-reactive antigen and the intensity of staining was similar to that seen in pretreatment biopsies. These results suggest that there was in vivo saturation of antigen sites at the higher doses of infused antibody. Additionally, there was no loss of expression of the KS1/4-reactive antigen and therefore no evidence for antigenic modulation.

Examples of carcinoma histopathology and immunoperoxidase stains are shown in Figs. 1–3. Nine sigmoidoscopic biopsies from patients treated with KS1/4 alone and immunoconjugate were examined within 24 h of completion of the 1661 mg total dose. In all of the biopsies, the colonic mucosa could
be immunostained with KS1/4. Bound antibody was detected in seven of the nine biopsies (see Fig. 4).

Pharmacokinetics and Human Anti-Mouse Response. To assess the circulating levels of murine MoAb achieved with the escalating dose regimen, serum levels of MoAb KS1/4 were measured at the start of each infusion and at 24, 48, and 72 h after completion of each infusion. With each dose escalation, postinfusion serum concentrations increased at each time point. The serum KS1/4 levels between the two groups at each dose did not differ significantly. Serum levels increased with each escalating dose of antibody at each time point [at the 24-h time point, \( \mu \text{g/ml} \) for KS1/4 alone: 50 mg, 0.58 ± 0.32 (SE); 100 mg, 3.48 ± 1.83; 500 mg, 4.60 ± 0.83; 1000 mg, 6.82 ± 0.93; and for KS1/4-methotrexate: 50 mg, 0.46 ± 0.15; 100 mg, 2.68 ± 0.51; 500 mg, 4.40 ± 1.07; 1000 mg 6 42 ± 1.53]. For each escalating dose of antibody administered, the serum KS1/4 levels achieved at 24 h declined over the ensuing 48 h (data not shown). In all patients there was a low background anti-mouse response that was present prior to initiation of treatment. Human anti-mouse response was found in both groups. A 2-fold increase in anti-mouse levels was detected within 3 weeks of treatment in five of six patients who received KS1/4 alone and in five of five patients who received KS1/4-methotrexate immunoconjugate (Table 1). Maximum levels were measured in the range of 0.4–2.0 mg/ml. The conjugation therefore did not appear to affect the immunogenicity of the KS1/4 antibody.

DISCUSSION

The antigen reactive with MoAb KS1/4 is expressed predominantly on epithelial malignancies and some normal epithelial tissues (1, 2). While the function of the antigen is unknown, its high density on the cell surface and homogeneous expression on non-small cell lung tumors suggested that it would be an excellent target for MoAb-drug therapy. We have previously shown that KS1/4-methotrexate immunoconjugate effectively inhibits the growth of adenocarcinoma of the lung cells, UCLA-P3, in vitro and suppresses the growth of established human lung adenocarcinoma xenographs in athymic mice (4). Recently, the sequence of cDNA clones that code for the KS1/4-reactive antigen has been determined (3, 6).

Murine monoclonal antibodies CO17–1A and GA733 produced to a human colorectal adenocarcinoma cell line and a gastric adenocarcinoma cell line, respectively, bind to carcinomas of the gastrointestinal tract and also bind in varying degrees to normal epithelial tissues. Both CO17–1A and GA733 immunoprecipitate a \( M, 40,000 \) glycoprotein and the sequence of the genomic clone for GA733 has recently been determined (7).

The predicted sequence for the antigens recognized by KS1/4 and GA733 has a greater than 50% homology and shows...
jugate when compared to the unconjugated MoAb, we did not
complex-mediated reaction in one patient who received anti
endobronchial mass histologically documented carcinoma.
ase, had received 6210 cGy to the lung and mediastinum and,
administration of MoAb 9.2.27 for the treatment of malignant
body alone. This patient developed fever, acute polyarthritis,
pruritus, urticaria, and anaphylaxis were not seen. The most
chills, anorexia, nausea, vomiting, diarrhea, mild anemia, and
occur following administration of the KS1/4-methotrexate con
conjugate KS1/4-methotrexate can be given to patients by i.v.
was expected that additional or more severe side effects would
was 28 mg. While it was expected that additional or more severe side effects would occur following administration of the KS1/4-methotrexate conjugate when compared to the unconjugated MoAb, we did not find this to be the case. Instead the same side effects (fever and chills, anorexia, nausea, vomiting, diarrhea, mild anemia, and brief increases in liver transaminases) were reported in both groups. These side effects are similar to those reported in other monoclonal antibody clinical trials (8-14) in which unconjugated monoclonal antibodies were used. Allergic reactions such as pruritus, urticaria, and anaphylaxis were not seen. The most significant toxicity encountered was a probable acute immune complex-mediated reaction in one patient who received antibody alone. This patient developed fever, acute polyarthritis, and circulating immune complexes after a total dose of 661 mg. At the time of reaction, human anti-mouse levels were measured at 1.8 mg/ml. Two other patients in this study had similar or higher levels without such systemic reactions. A similar clinical reaction characterized by fever and arthralgias and associated with circulating immune complexes has been described after administration of MoAb 9.2.27 for the treatment of malignant melanoma (15).

There was one possible clinical response in a patient who received KS1/4-methotrexate. This patient had Stage IIIIB disease, had received 6210 cGy to the lung and mediastinum and, prior to entry into the study, a directed biopsy of a visible endobronchial mass histologically documented carcinoma. After immunoconjugate treatment, the endobronchial mass appeared smaller, endobronchial biopsy showed only necrosis, and no histological evidence of viable carcinoma could be identified. This patient is alive and free of disease 43 months after treatment with KS1/4-methotrexate immunoconjugate.

Immunoperoxidase staining provided direct evidence for localization of both the MoAb and the MoAb-drug conjugate as well as activated complement fragments on the carcinoma cell membrane posttreatment. Biopsies taken during and after the course of immunotherapy showed selective localization and binding of monoclonal antibody KS1/4 to the carcinoma cell surface, confirming that antibody injected i.v. reaches carcinoma in vivo. Additionally, we found a dose-response relationship between binding of antibody to carcinoma and the quantity of i.v. administered antibody or immunoconjugate. Similar dose-dependent localization of MoAb to malignant cells has been described previously for anti-melanoma antibodies 9.2.27 (15) and R24 (13) and anti-T-cell antibodies (16, 17).

Posttreatment biopsies demonstrated deposition of C3d and C4c cleavage fragments on carcinoma cells corresponding to the sites of MoAb KS1/4 or MoAb KS1/4-methotrexate binding. Complement components deposit close to the site at which they are activated, and in this situation, the site of activation presumably is the antigen-antibody complex at the cell membrane. C3d and C4c deposition was found in both patient groups. Other MoAb clinical trials have described complement deposition in posttreatment biopsies of the targeted neoplasms. C3 deposition has been reported with 17–1A (9) and C3, C5, and C9 deposition has been reported with R24 (13).

Because MoAb KS1/4 cross-reacts with the mucosa of the colon, colonoscopic biopsies were taken to determine the presence of KS1/4 or its drug conjugate posttreatment. While the antibody could be detected in seven of nine colon biopsies from patients in both groups, it was not possible to correlate the presence of KS 1/4 alone or KS1/4-methotrexate with the observed gastrointestinal side effects.

Higher circulating serum levels of MoAb KS 1/4 were achieved with the administration of higher doses of antibody. We found no significant change in the serum levels of infused MoAb despite increasing levels of human anti-mouse antibodies over time. Similar observations have been made by some investigators (9, 18) but others have found that high circulating levels of anti-mouse antibodies were associated with low serum levels of circulating MoAb, decreased binding to tumor cells, and less
MONOCLONAL ANTIBODY AND DRUG CONJUGATE CLINICAL TRIAL

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REFERENCES

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Darlene J. Elias, Lynn Hirschowitz, Lawrence E. Kline, et al.