Phase I and Imaging Trial of a Monoclonal Antibody Directed against Gastrin-releasing Peptide in Patients with Lung Cancer

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ABSTRACT

Small cell lung cancer (SCLC) cells express and secrete bombesin-like peptides (BLP) that can activate specific receptors that stimulate the growth of these cells. A murine monoclonal antibody, 2A11, which binds to the BLP, gastrin-releasing peptide with high affinity, has been reported to decrease the growth of SCLC cells in vitro and in athymic nude mice. A Phase I trial in lung cancer patients was performed using multiple doses of 2A11. Thirteen patients with lung cancer received 12 doses of 2A11 antibody three times a week for 4 weeks at one of four dose levels. Serum samples were obtained prior to initiation and before each dose of 2A11 antibody therapy for measurement of 2A11 antibody levels and determination of serum human antimouse antibody levels. A pilot imaging evaluation using 111In conjugated 2A11 monoclonal antibody was also performed in the same patients to aid in the study of pharmacokinetics and biodistribution. No toxic reactions were observed, and none of the patients developed detectable human antimouse antibody; however, no objective antitumor responses were observed. The mean trough serum 2A11 levels in patients increased with increasing dose level: 0.26 ± 0.2 μg/ml, 6.7 ± 6 μg/ml, 71.5 ± 60 μg/ml, 248 ± 184 μg/ml for dose levels 1 mg/m², 10 mg/m², 100 mg/m², and 250 mg/m², respectively. At each dose level, sustained detectable serum levels of the monoclonal antibody were achieved. Tumor uptake was noted in 11 of 12 patients who were injected with 111In conjugated 2A11. Because no dose-limiting clinical toxicity was observed, a mathematical model was used to define the recommended Phase II dose of 250 mg/m². This trial established that repeated doses of monoclonal antibody 2A11 could be given safely to patients, and sustained levels could be achieved for a 4-week schedule. Further evaluation of the antitumor effects of 2A11 is warranted.

INTRODUCTION

There are 172,000 patients diagnosed with lung cancer each year (1), and ~16% of the cases are small cell histology. Patients with SCLC respond to combination chemotherapy >90% of the time with a typical median survival of 9–14 months (2). However, >95% of patients relapse and die of their SLCs. There is an urgent need for more effective approaches for lung cancer treatment.

Advances in the understanding of the biology of lung cancer have stimulated interest in the hypothesis of autocrine tumor stimulation by growth factors (3). The human BLPs, GRP, and neuromedin-B are produced and secreted by human SLCs (4–7). SLCs have also been found to express high-affinity receptors for these BLPs (8–10). The addition of BLPs to SLCs cells and bronchial epithelium grown in serum-free media stimulate DNA synthesis and clonal growth (11–13). The presence of an autocrine growth loop in SLC cells has been validated in vitro and in vivo experiments by using either the neutralizing monoclonal antibody 2A11 or peptide antagonists (13, 14). The binding specificity of the 2A11 monoclonal antibody has been characterized extensively, revealing that the antibody binds to the COOH-terminal region of the α-amidated, bombesin/GRP peptide. The same seven-terminal amino acids are required for receptor activation. When critical concentrations of the 2A11 monoclonal antibody bind GRP, the divalent antibody functionally sequesters the peptide, neutralizing the activation of the cellular receptor on lung cancer cells. The fact that GRP has a short biological half-life and is only rarely detected circulating in the blood of patients with extensive SLCs are favorable for this immunoneutralization strategy (15, 16). Athymic nude mice with xenografts of SLC cell lines were treated with 2A11 three times weekly for 4 weeks. At the completion of the study, the xenografts showed a >90% inhibition in tumor growth compared with mice treated with a control antibody that did not bind BLPs (13).

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The abbreviations used are: SCLC, small cell lung cancer; BLP, bombesin-like peptide; GRP, gastrin-releasing peptide; HAMA, human antimouse antibody; %ID/ml, percentage of injected dose/ml; AUC, area underneath the plasma curve; CT, computed tomography.

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Preclinical evaluation of this dosing schedule was conducted in a series of normal dogs, testing its physiological effect on gastric secretion and production. A gastroduodenal fistula was established in these dogs, which permitted serial evaluation of the consequence of the administration of the anti-GRP monoclonal activity on gastrin and acid production in the stomach. This experimental model permitted experiments that revealed that the single doses of up to 1 mg/kg (~20 mg/m²) of antibody were physiologically active in suppressing both the endocrine and paracrine effects of GRP on stomach gastrin and acidity (17). Higher doses (up to 2 mg/kg infused three times a week for 4 weeks) of monoclonal antibody were without any adverse clinical or pathological consequences. On the basis of these preclinical findings, a Phase I study of 2A11 was designed for patients with lung cancer. The patients were to be treated with the same schedule of 2A11 that was found to be effective in the xenograft model (three times a week for 4 weeks) and safe in normal dogs (17). The starting dose of 1 mg/m² was selected because this level has been well tolerated and used as a starting dose in other monoclonal antibody studies (18–20).

Because of the absence of toxicity at even the highest 2A11 dose used in the preclinical toxicology study, a model was developed to determine the serum concentration of monoclonal antibody that would be required to neutralize a range of observed concentration of GRP production from SCLC cell lines. The model predicted that a dose of 200 mg/m² would be required to reduce GRP receptor occupancy <10%, and this was based on a previous report that showed that this was the level of receptor occupancy that was the threshold for activation of GRP receptor signaling (21). Therefore, an antibody concentration of 200 mg/m³ every other day for 12 doses (2.4 g/m² per course) was predicted to be sufficient to neutralize GRP activation of its receptors on the SCLC cells. The objectives of the Phase I trial were to evaluate the toxicity of 2A11 in an antibody dose-seeking evaluation; document the achievable plasma concentrations; determine the pharmacokinetics of the 2A11 antibody; and finally to assess the frequency of HAMA formation.

Previous work had established that 2A11 could be conjugated to 125I without significant loss in immunoreactivity (22). Because 131I has some limitations as a radiotracer, including rapid dehalogenation and emission of high-energy gamma rays, we conducted the imaging evaluation using 11In-conjugated 2A11 monoclonal antibody. This conjugate was administered to patients in this study to facilitate the analysis of the biodistribution and pharmacokinetics of the 2A11 monoclonal antibody. We had reported previously that radiolabeled antibody could be safely administered endobronchially to dogs with efficient localization of the antibody to the mediastinal lymph nodes, and we did a pilot evaluation of intrabronchial injection of 11In-labeled 2A11 to determine whether this approach is feasible in humans (23).

MATERIALS AND METHODS

Patients. Patients with a histologically confirmed diagnosis of SCLC or non-SCLC were eligible to receive 2A11. Patients had to have progressed after receiving at least one chemotherapy regimen and not be candidates for potentially curative therapy. The studies performed prior to enrolling included history and physical examination, complete blood count, prothrombin time, partial thromboplastin time, serum chemistries, arterial blood gases, urinalysis, chest roentgenogram, electrocardiogram, computerized tomography of head and chest, and fiberoptic bronchoscopy with biopsy. The entry criteria were an Eastern Cooperative Oncology Group performance status of 0 to 3, (24) adequate hematological parameters included a total WBC count >2000/µL and platelet count >100,000/µL. Adequate renal and hepatic parameters included a serum creatinine of <2.0 mg/dl and a bilirubin <2.0 mg/dl Arterial blood gas parameters included pO2 >50 and pCO2 <50 with no history of myocardial infarction in the last 6 months, uncontrolled congestive heart failure, unstable angina, or uncontrolled arrhythmia.

Study Design. The development of the 2A11 murine monoclonal IgG1 antibody has been described previously (13). The antibody was produced, purified, and monitored in a fashion consistent with good manufacturing practice in accordance with the guidelines outlined in the Points to Consider memo from the Food and Drug Administration (25). The antibody was produced in amounts adequate for this therapeutic trial by Hybritech, Inc. and stored and distributed by the Cancer Treatment and Evaluation Program, National Cancer Institute, as part of a collaborative research agreement. Patients were treated with the unconjugated native antibody in a fashion to determine the maximal tolerated dose. The initial dose was 1 mg/m² of the monoclonal antibody in 5% human serum albumin, 150 mm sodium chloride, and 10 mEq sodium phosphate, at a pH of 7.3 given i.v. as an inpatient procedure in a 1–2-h period, three times a week for 4 weeks. Emergency support for anaphylaxis was available at the bedside. Vital signs were taken at 15-min intervals, and an i.v. line was maintained during, and an hour after, the antibody infusion. At least three patients were treated at each dose level. Both SCLC and non-SCLC patients were entered, but at least one patient at each dose had to have SCLC. In the absence of grade 2 toxicity, subsequent patients were entered at dose levels of 10 mg/m², the next three patients were to receive 100 mg/m², and the next three 250 mg/m² (18). There was no planned intrapatient dose escalation. The end point of dose escalation was demonstration of dose-limiting toxicity or achievement of pharmacologically guided end point for antigrowth factor therapy (26).

Serum samples obtained prior to initiation of 2A11 therapy and before each dose and were stored at ~70°C prior to measurement of 2A11 antibody levels using a solid-phase assay (17). Serum HAMA levels were also determined prior to each dose of 2A11 using a solid-phase antigen capture assay (18) and expressed as a ratio (HAMA index) of the posttreatment to pretreatment titers. If patients demonstrated an elevated HAMA titer more than three times baseline, the patients were not treated with further antibody administrations. If patients developed a positive HAMA titer, further analysis would be performed to evaluate the presence of anti-idiotypic host response (18).

Chest radiographs were performed weekly on therapy, and repeat bronchoscopy was performed at the end of 4 weeks if originally positive for tumor. Criteria for patients leaving the study included unacceptable toxicity on two occasions or any

grade IV toxicity, tumor progression, lack of tumor response after 4 weeks of 2A11, patient noncompliance, or refusal. The patients had their tumors assessed during their treatment course and at the end of their 4 weeks of treatment. Standard response criteria were used (27).

Imaging Studies: Radiolabeling. All patients were intended to receive a dose of radiolabeled 2A11 with their first and last dose of the monoclonal antibody. The GRP antibody was conjugated to diethylenetriamine pentaacetic acid using the mixed anhydride method (28). The antibody was provided in kit form containing 2 or 4 mg of the conjugated 2A11 and 2.2 mg of human serum albumin in a 2-ml volume (Hybritech, Inc.). For each study, up to 5 mCi of $^{111}$In-labeled citrate (Hybritech, Inc.) was added to a vial of chelate conjugate 2A11. After a 30-min incubation, quality control was performed using an Instant TLC method in methanol in water and showed 91 ± 11% protein bound activity (mean ± SD; Ref. 29). No further purification was performed. Patients received a mean of 4.4 + 0.9 mCi of $^{111}$In-labeled 2A11. As the dose of 2A11 antibody was escalated, unchelated 2A11 (1 mg/1 ml) was added to the radiolabeled $^{111}$In-labeled 2A11 to the desired final dose of 1, 10, 100, and 250 mg/m². Intravascular kinetics were determined by counting $^{111}$In radioactivity in plasma aliquots using a gamma counter. The %ID/ml was obtained by comparing the counts to a standard of the injected dose. The plasma volumes were estimated at the time of treatment using a normogram based on body surface area (30). Using the latter estimated volumes and the %ID/ml, the total percentage of the injected dose in the plasma volume was estimated. The monoclonal antibody infusion time for patients was short compared with the disposition half-life ($T_{1/2}$), so the intravascular data were treated similar to an i.v. bolus. The %ID/ml was fitted to a biexponential function to obtain both the α and β phase $T_{1/2}$ using a least-squares fit algorithm (SigmaPlot; Jandel Scientific, Duarte, CA). When tumor tissue was accessible and the patient gave permission, a local biopsy was performed to count the amount of radioactivity contained within a tumor nodule. These biopsies were performed as close to the day of isotope administration as possible.

Conventional pharmacokinetics parameters were then derived (31). The AUC was calculated in two steps. The AUC from the end of the antibody infusion ($T_{eq}$) to 168 h was obtained by trapezoidal integration of the decay-corrected plasma data; then, the terminal AUC was estimated using the terminal clearance rate to extrapolate from the activity retained at the last measured time point. Whole-body clearance of $^{111}$In was also determined over a 1-week interval using a sodium iodide gamma probe at a fixed distance of ~7 meters from the patient, taking the initial measurement after injection as 100%. Images were acquired using a gamma camera with a medium energy collimator and a 20% window centered at 174 and the 247 keV photopeak of $^{111}$In. Typically, whole-body and spot images were obtained in up to five occasions over a 7-day period and infrequently at later times. An experienced nuclear medicine physician reviewed imaging studies. Positive uptake in the tumor imaged was defined as radionuclide emission above adjacent background (32).

On the basis of pilot imaging data in dogs that showed a 2-log increase in specific radiolabeled antibody in draining regional lymph nodes compared with control antibody, we explored the feasibility of locoregional delivery of $^{111}$In-labeled 2A11 by bronchoscopic injection (23).

RESULTS

Patient Characteristics and Toxicity. Patient characteristics are summarized in Table 1. Most patients (13 of 15) had a diagnosis of SCLC. There were 11 males and 4 females enrolled. The median age was 59 years, with a range of 46–70 years and a median time from initial diagnosis of 18 months (7–38 months). Most patients had received at least two courses of prior chemotherapy, and two patients received three courses of chemotherapy before enrolling in the study. Thirteen of the 15 patients had achieved at least a partial response to chemotherapy. Among the 13 patients who had SCLC, the period of time from their last chemotherapy ranged from <1 month to 17 months. Five of these patients were enrolled in the study <3 months after their last course of chemotherapy. Two of these five patients had a partial response to their last course of chemotherapy, whereas the other three had progressive disease before enrolling in the trial. None of the patients had brain metastases, and five patients had received prophylactic cranial irradiation as prior therapy.

Four patients were treated with 2A11 antibody at a dose level of 1 mg/m², three patients at 10 mg/m², five at patients received 100 mg/m², and three patients at 250 mg/m². Two patients at dose level 100 mg/m² received only three doses of the antibody, because of progressive disease while on study. The other patients were treated with 12 doses of anti-GRP antibody. There were no detectable allergic reactions during the antibody administration. One patient developed thrombocytopenia with a platelet count of 26,000 while being treated with 2A11 at a dose level of 1 mg/m². Although the platelet level was >100,000 prior to enrollment into the study, this patient subsequently developed a platelet count of 73,000 during the first week of concomitant chest radiotherapy. In this patient only, limited

<table>
<thead>
<tr>
<th>Table 1 Clinical characteristics of 15 patients with lung cancer treated with escalating doses of 2A11 monoclonal antibody</th>
</tr>
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<td>Gender</td>
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<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Initial diagnosis</td>
</tr>
<tr>
<td>Non-SCLC</td>
</tr>
<tr>
<td>Prior courses of chemotherapy</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Sites of prior radiotherapy</td>
</tr>
<tr>
<td>Prophylactic cranial irradiation</td>
</tr>
<tr>
<td>Bone</td>
</tr>
<tr>
<td>Median time from initial diagnosis (time to enrollment in study)</td>
</tr>
</tbody>
</table>

*ECOG, Eastern Cooperative Oncology Group.*
field chest radiotherapy was instituted during week 2 of antibody administration to relieve occlusion of the left bronchus detected before starting the antibody and subsequent infiltrate detected during the first week of therapy. Because evaluable disease was evident outside to the radiation port, the patient was maintained on the antibody infusions. One patient developed grade 1 gastrointestinal symptoms of nausea and vomiting and chills that were self limited and not related to the timing of the antibody administration. For all of the other patients, the antibody administration was well tolerated without any detectable anaphylactic reactions or hematological, hepatic, or renal toxicities.

The two patients who received only three doses of the antibody progressed during treatment. One was diagnosed with brain metastases, and the other patient died of respiratory failure secondary to intrathoracic tumor progression. All patients but one died from progressive disease within a median time of 4 months (range, 1.5–17 months) after enrolling in the study. One patient lived for >2 years. He was originally diagnosed with limited stage SCLC and lived for 27 months after enrolling in the study. This patient had a slow pace of progression and was responsive to courses of radiation therapy to head and spine and then to ribs after going off study. No significant antitumor responses were seen during the course of this study.

Imaging Studies. All 15 patients who entered the study were injected with a mean of 4.4 mCi of 2A11 conjugated to 111In with their first dose of antibody. Tumor uptake was noted in 12 of 13 who had evaluable serial images (repeat imaging study at the time of the twelfth and final dose of monoclonal antibody). Two patients were considered not evaluable because they did not complete the month of therapy (both received only three doses of antibody). At the initial scan, which was obtained as soon after the initial antibody injection as feasible, the images showed predominantly intravascular activity including liver, blood pool, and renal uptake (Fig. 1A). The blood pool cleared during days 3–7 and tumor uptake increased relative to the background activity (Fig. 1B). The corresponding CT scan of the intrathoracic involvement of the same patient is shown in Fig. 1C. In addition, liver accumulation and bone marrow uptake of 111In was more prominent during days 3–7. A summary of the results of the imaging studies with the 111In-labeled 2A11 is compared with the CT imaging results (Table 2). As shown in Table 2, there is thoracic uptake of 111In-labeled 2A11 in the 12 of 13 patients. The thoracic uptake corresponded to a discreet lesion on CT scan in 11 cases. In the two discordant cases, one patient had NSCLC histology, and the other case showed increased radioactivity in an area of previous chest radiation. Local biopsy of tumor in peripheral lymph nodes was possible in three patients (Fig. 2), and the total concentration of labeled antibody is included in Table 2. The recovered activity ranged from 0.004, 0.0017, to 0.001% of the ID/g of tumor tissue from biopsies obtained 3, 4, and 8 days after the injection of 111In-labeled 2A11, respectively.

Two patients were studied with intrabronchial injection of the 111In-labeled 2A11 at the completion of their course of serotherapy. The 111In-labeled 2A11 was delivered submucosally at time of bronchoscopy using an aspiration cytology catheter as described previously (23). Prompt uptake was detected in regional thoracic lymph nodes. In one patient, a small amounts of activity was also promptly detected in the region of the stomach, presumably from the swallowing of radiolabeled material cleared from the injection site by coughing. No untoward consequences of the intrabronchially imaging studies were noted.

A summary of circulating monoclonal antibody terminal half-life ($T_{1/2}$) and total AUC at different dose levels are shown in Table 3. Extensive pharmacokinetic data were available for 12 patients treated with 2A11. Three patients were not included because adequate curve fitting was not possible. Five patients had paired studies performed within a mean of 18 days (range, 14–21 days) apart. The mean AUC of the 111In radio-labeled antibody at different dose levels of native antibody was similar, ranging from a mean of 2.25%ID × h/ml for the 1 mg/m² dose to 2.33%ID × h/ml for the 250 mg/m² dose (ANOVA, $P = 0.266$; Table 2). The terminal half-life ($T_{1/2}$) of the radiolabeled antibody was also similar, ranging from a mean of 51.8–63.6 (ANOVA, $P = 0.269$). The five patients with paired studies did not show any difference in the AUC ($P = 0.248$) or terminal half-life ($P = 0.844$) between the first and second injections.

Pharmacokinetics. Thirteen patients treated with at least 11 doses had serum collected and antibody levels determined. The mean trough serum level of 2A11 antibody increased with increasing dose level in patients treated with 2A11. The trough serum level of 2A11 antibody was $0.26 + 0.2 \mu g/ml$, 6.7 + 0.6 yu/ml, 71.5 + 60 yu/ml, and 248 + 184 yu/ml for dose levels 1, 10, 100, and 250 mg/m², respectively. At each dose level, a sustained level of monoclonal antibody was achieved (Fig. 3). The ratio of posttreatment to pretreatment HAMA titers, as determined by the solid-phase assay, was < 1 in all patients at all dose levels. No anti-idiotypic antibody formation was observed in this study population.

DISCUSSION

A large number of monoclonal antibodies have been generated against a wide variety of tumor-associated antigens. Growth factor receptors and oncogene products are an interesting class of tumor-associated antigens. In this trial, the monoclonal antibody is directed against the growth factor itself and not its receptor. From the clinical trials to date with monoclonal antibodies directed to targets with the growth factor receptor circuits, it is not clear what the relative merits of targeting the ligand are, compared with targeting the receptor to block the growth factor effect. In experimental systems such as with the transferrin-directed immunological reagents, antibodies to the receptor were equipotent to the antibodies to the ligand (33). The basis for this therapeutic approach was to deliver sufficient anti-GRP monoclonal antibody into the tumor bed so as to bind all available GRPs before the peptide can bind to the tumor cell receptors. This trial began in 1988, and the Phase II trial is still ongoing. Logistical issues relating to the production of sufficient antibody (>100 g) to complete the Phase II trial after observing a response to therapy have caused a protracted delay in completing the study. The original intent was to report the results of the Phase I and II trials simultaneously. Recently, a trial with a similar strategy to interrupt a growth factor circuit has been reported with the anti-p185HER2 monoclonal antibody. The
remarkable response rate and survival duration of the combination of chemotherapy with the anti-p185HER2/neu monoclonal antibody has generated a renewal of interest in monoclonal antibody-based therapies. When used as a single agent, the Phase II study of weekly i.v. anti-p185HER2/neu monoclonal antibody in patients with HER2/new overexpressing metastatic breast cancer was reported to have an overall response rate of 11.6% (34). The antibody to GRP has already been associated with a complete response in the initial part of the Phase II trial that has been reported, so that the single-agent complete response rate is comparable for the two antibodies (35). Potentially, 2A11 will provide an immunological reagent to test how robust the favorable interaction is between the coadministration of chemotherapy with a monoclonal antibody that neutralizes a salient growth factor pathway. In light of the protracted delay in completing the Phase II trial, along with the renewed interest in the use of monoclonal antibodies to block growth factor circuit, we felt compelled to report this experience at this time. Because completion of this study has taken so long, the occurrence of a complete response of a patient treated on the Phase II trial has been already reported (35). This decision was made because of the uncertainty about the availability of sufficient antibody to complete a statistically valid Phase II trial. Because ~6 g of antibody are required for each Phase II patient, the cost of the pharmaceutical grade monoclonal antibody is a daunting issue. The fact that there was no discernable toxicity with this therapy and that the mouse antibody infusions did not result in significant host response both suggested the need to complete a full Phase II experience with this novel reagent.

In this translational research effort to explore for a new approach to arresting the growth of SCLCs, several trial design features are of interest. To be successful with monoclonal antibody-based immune neutralization of an autocrine growth factor in a cancer, a saturating excess of monoclonal antibody had to be delivered to the tumor bed during therapy. Assuming the highest recorded GRP peptide production (10–30 nM) as well as the highest reported density of GRP receptors on SCLC cell lines (1–100 nM), 200 mg/m² of monoclonal antibody 2A11 should be sufficient to successfully compete with the tumor-produced GRPs and inhibit tumor cell mitogenesis (21). The proof of concept of this analysis was provided by the case report of the initial Phase II patients treated with this antibody (35). In a similar Phase I trial of 111In-labeled anti-epidermal growth factor murine monoclonal antibody 225, groups of three patients received a single total dose ranging from 1 to 300 mg, and tumors were imaged in all patients who received doses 20 mg or greater (19). No toxicity was observed, but human anti-murine antibodies were found in all patients by day 8. Another Phase I trial evaluated the murine monoclonal antibody CO17-1A, which is directed against Mr 37,000 glycoprotein present on the cell surface of gastrointestinal cancers. Repeat doses of monoclonal antibody were well tolerated. Of the 15 patients who received more than one infusion of 17-1A at weekly intervals over 1–4 weeks, 6 had antibody to 17-1A prior to the second infusion, whereas 4 of 5 had antibody detectable prior to the third infusion (36). Repeated administration of murine monoclonal antibodies is frequently limited by the development of HAMA, resulting in rapid clearance of the murine antibody from the circulation (37). In this trial, HAMA as detected by

Fig 1  Radionuclide and computerized images from a patient with the diagnosis of SCLC. The patient was treated with 5 mCi of 111In-labeled 2A11 and 210 mg of the 2A11 antibody. Images were obtained serially on days 1–8. A, the anterior view of the chest on the day of injections shows predominantly blood pool activity with the later images (B) showing clearance of the blood pool with retention of activity in the mediastinum. In addition, marked uptake in the liver is evident, which is characteristic of 111In conjugates. Corresponding radiograph study of the same patient done prior to enrolling in the study is also shown. C, computerized tomography of the chest prior to treatment showing mediastinal adenopathy (arrow).
immunological assay did not develop in any of the patients receiving antibody at all dose levels. The serum levels of 2A11 antibody remained relatively constant through the course of treatment with repeated doses of antibody administration, indicating that significant HAMA was indeed not present. This finding contrasts with the usual experience with repetitive mouse antibody dosing in an immunocompetent subjects that often results in HAMA formation (37, 38). The absence of development of HAMA may result from the suppressing effect of continuous large doses of 2A11. Other workers have also observed this in a pharmacokinetic assessment of various treatment schedules (39). The treatment schedules that were studied included intervals of 6 weeks between treatments or every 2-day infusions for up to 8 weeks. When infusions were given every second or third day, the IgG response was delayed by 30–50 days and remained at a lower level. Peak titer was noted 70–110 days after institution of therapy. The inhibitory effects of massive monoclonal antibody administration in this study were also shown to be temporary, because the one patient that was reexposed to 2A11 developed a measurable antibody response (35). We did not assess patients for the development of HAMA after the course of antibody was completed, although this may have

Table 2  Summary of 111In-labeled 2A11 antibody imaging results with other relevant parameters

<table>
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<tr>
<th>Patient Number</th>
<th>2A11 image</th>
<th>CT image</th>
<th>Histology</th>
<th>Prior CTR</th>
<th>111In-labeled 2A11/g tumor tissue</th>
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<tr>
<td>1</td>
<td>†R lung uptake</td>
<td>RUL lesion</td>
<td>SCLC</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>R&amp;L hilar/mediastinal adenopathy</td>
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<td>6</td>
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<td>SCLC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>†mediastinal uptake</td>
<td>4 × 6 cm mediastinal mass</td>
<td>SCLC</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>†L lung uptake</td>
<td>L lung lesion</td>
<td>SCLC</td>
<td>Yes</td>
<td>35 pm</td>
</tr>
</tbody>
</table>

CTR, chemotherapy.
†, increased; †R, right; SCLN, supraclavicular lymph node; RUL, right upper lobe; R&L, right & left; L, left; RLL, right lower lobe.

Fig. 2 Photomicrograph of a histological section from a submandibular node biopsy obtained after therapy, demonstrating SCLC in the same patient as Fig. 1 (H&E; ×250).
more relevance when contemplating retreatment. A significant factor, which may have contributed to the lack of HAMA formation seen in this study, may be attributable to the absence of immune complex formation. This may be because GRP is a monovalent target for 2A11. The absence of HAMA in response to 2A11 administration may obviate the need for alteration of the antibody through chimerization or humanization.

The most important observation in this trial was the lack of toxicity in the patients who received high doses of 2A11 antibody. Because no dose-limiting clinical toxicity was observed, the standard practice of defining the maximally tolerated dose was not feasible. As a result, this trial is a Phase I trial guided by achieving an optimal biologically dose of the therapeutic agent. Documenting that sustained levels of 2A11 antibody were achieved with repeated administration was an important goal in our trial. A serum level of \(100 \text{ mg/ml}\) was achieved at the dose level 250 mg/m\(^2\). This was 10-fold greater than the predicted 10 mg/ml concentration required for growth inhibition (21). Similar antibody levels have been seen in pharmacokinetic determinations of other murine monoclonal antibodies. In studies of humanized anti-p185HER2/neu monoclonal antibody, weekly doses achieved a target trough serum concentration of 10–20 mg/ml, which was also associated with antitumor activity in preclinical models (34). In the pharmacokinetic study of various treatment schedules of murine monoclonal antibody 17-1A, nadir values ranging from 6 to 60 μg/ml were achieved with repeated infusions on Mondays, Wednesdays, and Fridays for 8 consecutive weeks. The ability to achieve high and sustained levels of the growth factor neutralizing antibody 2A11 may be fundamental to the success of this therapeutic strategy.

Recent studies in the literature have also demonstrated the diagnostic utility of radiolabeled monoclonal antibodies directed against a variety of tumor-specific and oncofetal tumor antigens (40–42). In this study, primary or metastatic lesions could be detected by \(^{111}\text{In-labeled anti-GRP antibody in 11 of 13 evaluable cases. Radiolabeling also provided valuable information regarding clearance and biodistribution of antibody. The antibody exhibited first-order kinetics with no evidence of dose-dependent changes observed in the biodistribution of doses ranging from 1 to 250 mg/m\(^2\). In addition, imaging and biodistribution studies showed similar kinetics for the initial dose and that administered within a mean of 18 days (range, 14–21). Because tumor acquisition was not routinely possible without a major thoracic surgical procedure, we were only able to specifically measure the percentage of injected antibody dose that was retained in tumor tissue in three patients. The observed concentration range of \(^{111}\text{In-labeled 2A11 of 0.001–0.004% of injected dose recovered per gram of tumor measured in the three biopsy specimens is consistent with the expected concentrations discussed in the original modeling analysis (21). It is interesting to note that the patients who received recent chest radiation therapy had increased antibody retention diffusely in the area of recent radiation therapy. We assumed that this was because of an endothelial injury of the blood vessels in the radiation port that appeared to increase the leak of the radiolabeled antibody into the lung tissue. This possibility suggests a potential way to increase antitumor response by interference with autocrine loops. Jain and co-workers (43) recently studied the effect of radiotherapy on the vascular permeability and showed that there was a significant increase in the effect of

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters derived from blood counting data in (^{111}\text{In-labeled 2A11 nuclear medicine study.}</th>
<th>1 mg/m(^2)</th>
<th>10 mg/m(^2)</th>
<th>100 mg/m(^2)</th>
<th>250 mg/m(^2)</th>
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</thead>
<tbody>
<tr>
<td>Total AUC (% ID × h/ml)</td>
<td>2.25 ± 0.22</td>
<td>2.43 ± 1.0</td>
<td>3.10 ± 0.52</td>
<td>2.33 ± 0.31</td>
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<tr>
<td>(T_{1/2, a}) (h)</td>
<td>0.71 ± 0.7</td>
<td>3.22 ± 7.4</td>
<td>2.99 ± 0.75</td>
<td>1.19 ± 0.75</td>
</tr>
<tr>
<td>(T_{1/2, b}) (h)</td>
<td>51.82 ± 10.94</td>
<td>64.23 ± 2.3</td>
<td>60.19 ± 7.01</td>
<td>63.66 ± 4.97</td>
</tr>
</tbody>
</table>

**Fig. 3** Serum 2A11 levels in 13 patients at all dose levels that received 12 doses of the antibody 2A11. Serum obtained prior to each dose was assayed using a solid-phase assay.
an anti-VEGF monoclonal antibody, possibly attributable to the increased leakage of the monoclonal antibody into the tumor bed. Jain also recently reviewed the challenges in delivering blood-borne agents to a tumor bed, and these factors may contribute to the infrequent success of this anti-GRP antibody strategy (44). In contrast, it is tempting to speculate that the unexpectedly good results with Taxol with the anti-p185HER2/Neu monoclonal antibody may be attributable to the increased tumor penetration of the antibody because of the endothelial injury mediated by a xenobiotic stress (34). In the setting of disrupted vasculature, a tumor cell may be more reliant on its microenvironment for growth factors, and yet the increased percolation of the antibody in this setting of leaky vasculature allows for more antibody to be available to neutralize the locally produced growth signals. Further evaluation of the possibility of chemotherapy administered in combinations with 2A11 to establish whether anti-growth factor/chemotherapy combinations are of general utility is under consideration.

Another reason why a tumor could be infrequently unresponsive to growth factor-directed therapy such as with anti-p185HER2/Neu or anti-GRP monoclonal antibody is because of the ability of cancer cells to produce multiple autocrine growth factors. We showed recently that autocrine stimulation of lung cancer cells for both IGF-1 and GRP could be interrupted by the use of lipoxygenase inhibitors (45). Blocking autocrine growth factor stimulation by targeting conserved signal transduction pathways is another alternative to deal with our evolving knowledge of the complexity of autocrine growth circuits in advanced cancer cells. Finally, the preliminary findings with intrabronchial delivery of radiolabeled antibody from this study, coupled with the published results of this approach with the dogs, suggest that locoregional delivery strategies may share the pharmacological advantage seen with regional delivery of antibodies in other cancer applications (23, 46). Further work with regional drug delivery in the lung using aerosols is ongoing to determine whether this type of delivery should become the preferred way to administer pharmaceuticals. This delivery approach could be especially relevant with the early stages of lung cancer, where autocrine growth circuits may be less extensive than with advanced cancer (47).

In conclusion, this is a Phase I study that was completed without encountering discernable toxicity but with achieving an intravascular level of monoclonal antibody predicted to be sufficient to block GRP-mediated tumor cell stimulation. Completion of the Phase II trial at the monoclonal antibody dose of 250 mg/m² is under way to define the response rate associated with the growth factor-directed monoclonal antibody approach. Further work is warranted to find clinical situations where neutralizing the effects of growth factors on cancer progression is useful.

ACKNOWLEDGMENTS

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Phase I and Imaging Trial of a Monoclonal Antibody Directed against Gastrin-releasing Peptide in Patients with Lung Cancer

Arvind Chaudhry, Jorge A. Carrasquillo, Ingalill L. Avis, et al.


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