ING-1, a Monoclonal Antibody Targeting Ep-CAM in Patients with Advanced Adenocarcinomas

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ABSTRACT

INTRODUCTION

Purpose: To determine the feasibility of administration, safety, toxicity, immunogenicity, pharmacokinetics, maximum tolerated dose, and biodistribution of ING-1, a high-affinity, Human-Engineered monoclonal antibody (heMAb) to the Mr 40,000 epithelial cell adhesion molecule Ep-CAM, in patients with advanced adenocarcinomas.

Experimental Design: ING-1 was initially administered to patients as a 1-hour intravenous infusion every 3 weeks. Toxicity and pharmacokinetic data led to the evaluation of a weekly schedule. The distribution of iodine-131 (131I)-labeled ING-1 was studied.

Results: Twenty-five patients received 82 courses of ING-1. Minimal toxicity was initially observed at the 0.03-, 0.10-, and 0.30-mg/kg dose levels. A patient dosed at 1.0 mg/kg developed acute pancreatitis with severe abdominal pain, nausea, and vomiting. A patient dosed at 0.3 mg/kg had an asymptomatic amylase and lipase elevation to 502 units/L and 1,627 units/L, respectively. Both patients made uncomplicated recoveries. No other dose-limiting toxicities were observed. Regardless of dose, the volume of distribution (mean ± SEM) was 46.6 ± 1.6 mL/kg. ING-1 clearance decreased with increasing dose. To minimize toxicity and increase dose intensity, we then administered ING-1 weekly.

Conclusion: The recommended dose for ING-1 is 0.10 mg/kg by intravenous infusion weekly. The absence of severe toxicity at this dose, low immunogenicity, and preliminary evidence of ING-1 tumor localization and antitumor efficacy support the further clinical development of this antibody to treat Ep-CAM–positive malignant diseases.

ING-1 is a high-affinity, Human-Engineered monoclonal antibody that recognizes Ep-CAM [also known as 17-1A antigen, (1) KSA, (2) EGP-2, (3) EGP40 (4), or GA733–2 (5)], a Mr 40,000 glycoprotein that functions as a homotypic epithelial cell adhesion molecule (6). Ep-CAM negatively regulates cadherin-mediated cell adhesion, decreasing its association with the cadherin/β-catenin complex, which inhibits differentiation and promotes proliferation (7–11). Ep-CAM expression portends tumor differentiation and is an independent predictor of survival in breast cancer (10, 12–14). Highly expressed on the surface of many adenocarcinomas, ~85% of colorectal carcinomas express Ep-CAM, with more than 80% of tumor cells expressing >106 molecules/cell (15, 16). Ep-CAM is also expressed on some normal tissues, but the density of antigen expression is much higher on tumor cells (17). Studies in transgenic mice expressing Ep-CAM under Ep-CAM-specific regulatory sequences also indicate that Ep-CAM expression in normal epithelial tissue is not as accessible as tumor cell Ep-CAM, (18), which suggests that the therapeutic targeting of Ep-CAM may have a favorable therapeutic index. Monoclonal antibodies to Ep-CAM induce tumor regression in xenograft studies, with higher-affinity antibodies having superior antitumor activity to lower-affinity antibodies (19, 20).

Edrecolomab (Panorex, Glaxo Smith Kline, Research Triangle, NC) is a low-affinity, murine monoclonal antibody to Ep-CAM. Clinical trials indicate that it is well tolerated, although it has limited antitumor efficacy (21–27). Nonetheless, in a randomized study of patients with Dukes’ C colorectal cancer (28), edrecolomab decreased tumor-related mortality by 32%. However, a three-arm randomized study comparing adjuvant 5-fluorouracil (5FU) and leucovorin (LV) with the combination of edrecolomab and 5FU and LV, and edrecolomab alone reported no survival advantage from edrecolomab therapy (29). A second randomized trial, comparing edrecolomab in combination with 5FU-based chemotherapy to 5FU-based chemotherapy alone, suggested a small improvement in overall survival for...
PATIENTS AND METHODS

Patient Selection. Patients who had solid malignant disease and whose disease was refractory to standard therapy were eligible for this phase I study. Tumor cell Ep-CAM expression was not mandatory for participation. Eligibility required the following: age ≥18 years; life-expectancy of at least 12 weeks; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; no prior chemotherapy within 4 weeks; adequate hematopoietic function [hemoglobin ≥9g/dL, absolute neutrophil count (ANC) ≥1,500/μL, platelet count ≥100,000/μL], hepatic function [bilirubin ≤1.5 mg/dL, aspartate serum trans‐ferase (AST), alanine serum transferase (ALT), and alkaline phosphatase at least three times the upper limit of normal, or at least five times institutional upper limit of normal if the elevation were due to hepatic metastases], and renal function (serum creatinine ≤1.5 mg/dL); measurable or evaluable disease; no evidence of brain metastases; and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients gave written informed consent for all clinical and research aspects of the study, according to federal and institutional guidelines before treatment.

Study Design, Dosage, and Drug Administration. ING-1 was supplied as single-use vials containing 50 mg of sterile ING-1 at a concentration of 5 mg/mL in 20 mmol/L phosphate, 150 mmol/L sodium chloride (NaCl), and 0.005% polysorbate 80, with a pH of 6.5. ING-1 was mixed in 50 or 100 mL of 0.9% NaCl. It was stored under refrigeration at 2°C to 8°C and was prefiltered with a 0.22-μm, low-protein-binding, filter. ING-1 was initially administered as a 1-hour intravenous infusion every 3 weeks. Toxicity and pharmacokinetic data then led to the study of a weekly schedule. The dose levels to be tested were 0.03, 0.1, 0.3, 1.0, and 3 mg/kg.

Toxicity was graded by the Common Toxicity Criteria of the National Cancer Institute (NCI-CTC), Version 2 (NIH, Bethesda, MD). Dose limiting toxicity (DLT) was defined as any of the following occurring during the first treatment course: (a) NCI-CTC grade II allergic reaction defined as symptomatic bronchospasm with or without urticaria or drug fever ≥38°C (100.4°F) in patients who had received optimal prophylaxis and treatment for this; (b) NCI-CTC grade III or IV vomiting or diarrhea in patients who had received optimal prophylaxis and treatment; (c) any other CTC Grade III or greater toxicity.

Cohorts of four patients were evaluated at each dose level. When DLT was encountered in one of four patients during the first course of therapy, two additional patients were to be enrolled at that dose level. If DLT was not observed in the additional patients, new patients were to be treated at the next higher dose level. When two patients in a cohort experienced DLT in their first course, this was defined as the toxic dose level. The maximum tolerated dose (MTD) was the dose below the toxic dose.

For the study of ING-1 biodistribution, the final cohort of patients also received, as part of the first dose of ING-1, 1 mg of iodine-131 (131I)-labeled ING-1 (10 mCi) administered after the infusion of unlabeled ING-1 over 4 minutes. Radiolabeling was performed on the day of administration under aseptic conditions with standard iodogen methodology, as described previously (34).

Pretreatment and Follow-up Studies. A complete medical history, physical examination, concurrent medication profile, assessment of performance status, and routine laboratory studies were done before treatment started and weekly. Routine laboratory studies included a complete blood count, differential white blood count, prothrombin and partial thromboplastin times, electrolytes, blood urea nitrogen, serum creatinine, uric acid, glucose, alkaline phosphatase, lactate dehydrogenase, ALT, AST, total bilirubin, calcium, total protein, albumin, amylase and lipase, and urinalysis. Pretreatment studies also included an electrocardiogram (ECG), relevant radiologic studies for the evaluation of all measurable and evaluable sites of disease, and an assessment of appropriate tumor markers. ECG examination was repeated every 3 weeks. Radiologic studies for disease status were repeated after every other course. Patients were able to continue treatment in the absence of progressive disease, which was defined as a 25% increase in the size of any lesion or appearance of any new lesion. A complete response was scored if there was disappearance of all disease on two measurements separated by at least 4 weeks. A partial response required at least 50% reduction in the sum of the product of the bidimensional measurements of all documented lesions separated by at least 4 weeks.

Pharmacokinetic Sampling and Assays. To study the pharmacokinetics of ING-1, were obtained whole blood samples from an indwelling venous catheter placed in the arm contralateral to the drug infusion. Samples were obtained before ING-1...
administration and, after the end of the first infusion, at 5 minutes; at 4, 24, and 72 hours; and on days 8 and 15. Samples were also collected prior to infusion and 5 minutes after the end of infusion for the next four doses. The samples were immediately placed in EDTA tubes, were inverted 10 times, were transported on ice to the laboratory, were centrifuged at 4°C at 1,200 × g for 5 minutes to separate plasma, and then were frozen at −70°C. Ing-1 plasma concentrations were determined by ELISA. Samples were quantified from a calibration curve prepared by adding ING-1 to human plasma. The proportion of recovered ING-1 was assessed by using a linear regression curve of measured ING-1 concentration versus added ING-1 concentration. The calculated slope was used as the fractional recovery, which ranged from 0.572 to 0.964 for this study. The plasma concentrations of ING-1 were corrected for fractional recovery.

Plasma samples for measuring the antibody response to ING-1 were also collected prior to treatment and on days 22, 43, 64, and 84 and were assessed by ELISA. The assay used bound ING-1 antibody as the target antigen, and biotin-labeled ING-1 antibody and alkaline phosphatase-labeled streptavidin (Zymed Laboratories, South San Francisco, CA). The detection limit for this assay was ~10 ng/mL.

**Pharmacokinetic Analyses.** Population pharmacokinetic analyses were performed with NONMEM (nonlinear mixed-effect modeling software), and a one compartment model [first-order conditional estimation method (FOCE), NONMEM Project Group, University of California at San Francisco, San Francisco, CA):

\[
\frac{dX}{dt} = -\frac{CL}{Vc}X + R
\]

\[
C = \frac{X}{Vc}
\]

where \( R \) is the rate of intravenous ING-1 infusion in \( \mu g/kg/d \), \( C \) is the predicted plasma concentration of ING-1 in \( \mu g/mL \), \( Vc \) is the volume of distribution in mL/kg and \( CL \) is the clearance in mL/kg/d.

It was assumed that each pharmacokinetic variable was log-normally distributed among the subjects about a population mean. \( CL \) decreased with increasing dose, which suggested that it was saturable, but it did not change sufficiently to support a Michaelis–Menten model. Therefore, a linear covariate model in the logarithmic domain was used to model the change in \( CL \) with dose level:

\[
\log(CL_i) = \log(CL_{interecpt}) + CL_{slope}\log(Dose \ level) + \eta_{CLi}
\]

\[
\log(Vc_i) = \log(Vc) + \eta_{Vci}
\]

for subject \( i \). \( Vc \) was the population mean volume of distribution of the central compartment; \( CL_{interecpt} \) and \( CL_{slope} \) were the population covariates describing the change in \( CL \) with dose; and \( \eta_{CLi} \) and \( \eta_{Vci} \) were the intersubject random deviates of \( CL \) and \( Vc \), respectively, about the population mean for subject \( i \).

The intrindividual error was modeled by using a mixed heteroscedastic and homoscedastic function:

\[
\text{Variance} = (\sigma_p C + \sigma_c)^2
\]

where \( \sigma_p \) is the proportionate error coefficient, and \( \sigma_c \) is the constant error coefficient. The constant error coefficient was included to take into account the assay background observed in the data. The raw data were fitted with the above intrindividual error model, which accommodates the constant measurement error at low concentration levels, and the proportional measurement error that predominates at higher levels, typical of assays that use serial dilution techniques. After the population analysis was performed, post hoc analysis for each subject was performed to obtain individual pharmacokinetic analytes. Secondary descriptive pharmacokinetic parameters were calculated from these individual parameters as follows:

\[
C_{max} = \frac{\text{Dose level (in mg/kg)}}{Vc_i}
\]

\[
AUC_i = \frac{\text{Dose level (in mg/kg)}}{CL_i}
\]

\[
T_{\frac{1}{2},in} = \ln(2) \frac{Vc_i}{CL_i}
\]

**Quantitative Imaging Techniques.** Planar conjugate views were acquired with a Philips Axis dual-detector camera that was interfaced to an Odyssey Nuclear Medicine Imaging system (Philips Medical System, Cleveland, OH). Before labeled-antibody injection, images were obtained with a Cobalt-57 sheet-source with or without the patient. Planar conjugate views were acquired immediately and at 4, 24, 48, 96, and 168 h after the administration of labeled-antibody. A 50-mL calibrated reference source containing 50 \( \mu Ci \) of \( ^{131}I \) was also imaged during the whole body scan. This reference source was used to determine the calibration factor, converting counts in region(s) of interest (ROI) to radioactivity. ROI were visually defined by the operator. Tissue background radioactivity was corrected by subtracting the counts in the background ROI. The geometric-mean method was used to quantify radioactivity for large organs (e.g., liver, lungs) clearly identified on conjugate views (35, 36). The effective-point-source method was used for objects that were clearly identifiable on only one view (37, 38). Radioactivity in tissue was expressed as \( \mu Ci \) and the percentage of injected radioactivity dose (%ID). The biological half-life (\( T_{\frac{1}{2},in} \)) of radioactivity in tissues was determined by fitting the %ID data at various time points to a monoexponential curve.

**RESULTS**

Twenty-five patients, whose pertinent demographic characteristics are displayed in Table 1, received eighty-two courses of ING-1. This was dosed as a 1-hour intravenous infusion every 3 weeks or weekly (3 of 7 patients in the 0.1 mg/kg dose group). The following dose levels were studied: 0.03 (\( n = 4 \)), 0.1 (\( n = 7 \)), 0.3 (\( n = 12 \)) or 1.0 (\( n = 2 \)) mg/kg. The median number of administered complete courses was two (Table 2). No significant toxicity was observed at the 0.03, 0.1, or 0.3 mg/kg dose levels. Dose escalation to 1.0 mg/kg was pursued. The second patient treated at this dose level developed acute pancreatitis with severe abdominal pain, nausea and vomiting, sweating and hypotension, immediately after completing the first infusion of ING-1. This patient made an uncomplicated recovery, and re-
Safety, Pharmacokinetics, and Biodistribution of ING-1

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>37–79</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
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<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Site of primary disease</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>1</td>
</tr>
<tr>
<td>Colorectal</td>
<td>15</td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>3</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
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<td>3</td>
<td>5</td>
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<tr>
<td>3+</td>
<td>16</td>
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<tr>
<td>Prior hormone therapy</td>
<td>4</td>
</tr>
<tr>
<td>Prior radiation</td>
<td>9</td>
</tr>
<tr>
<td>Prior surgery</td>
<td>23</td>
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</table>

Table 2  Dose escalation scheme

<table>
<thead>
<tr>
<th>No. of courses completed</th>
<th>No. of patients treated at dose level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.03 mg/kg (n = 4)</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7–24</td>
<td>0</td>
</tr>
<tr>
<td>All courses</td>
<td></td>
</tr>
</tbody>
</table>

received no further courses of ING-1. No additional patients were enrolled at the 1.0-mg/kg dosage level. The first patient enrolled at 1.0 mg/kg received all subsequent doses at 0.3 mg/kg. Enrollment was continued at 0.3 mg/kg, with 8 more patients being enrolled at this dose level. One of the additional patients treated at 0.3 mg/kg experienced asymptomatic increases of serum amylase (grade 3) and lipase (grade 4) levels 4 hours after completing course one. No other episodes of pancreatic enzyme elevation were observed.

The half-life of ING-1 in humans was shorter than that predicted from preclinical studies. Preclinical studies also indicated that the lowest biologically relevant concentration of ING-1 is 0.1 μg/mL. The plasma concentration of ING-1 in this clinical trial was above this for less than 7 days at 0.1 mg/kg. To sustain drug levels above this concentration, and to increase dosing intensity, we then administered ING-1 once a week at 0.1 mg/kg. No significant toxicity was observed in three patients, who were selected to have tumors that expressed high levels of Ep-CAM (+ + + as determined by immunohistochemistry) and who were given a tracer dose of 131I-labeled ING-1 to study the antibody biodistribution.

Toxicities

The most common ING-1–related adverse events were grade 1 or 2 asthenia, nausea, vomiting, diarrhea, fever, and chills (Table 3). These symptoms were generally short-lived, lasting less than 24 hours. The fever and chills resolved with antipyretics and antihistamines.

Pancreatitis. Two patients developed biochemical evidence of a rapid onset, and reversible, drug-induced pancreatitis. This commenced soon after infusion was completed, with rapid and spontaneous resolution. A 60-year-old 86-kg female with colorectal carcinoma metastatic to the lung, treated at 1.0 mg/kg, complained of abdominal pain with nausea and vomiting at the end of infusion. Her amylase and lipase levels increased from 70 and 34 units/L, respectively, at baseline, to 1,411 and 6,196 units/L at 4 hours postinfusion. The patient’s amylase and lipase levels returned to normal after 6 days, and she made a complete and uncomplicated recovery. The patient was not given ING-1 again. The second patient, a 54-year-old 94-kg female with colorectal cancer, was treated at 0.3 mg/kg and developed asymptomatic NCI-CTC grade 3 amylase and NCI-CTC grade 4 lipase, elevations during course one, increasing from 76 and 37 units/L, respectively, at baseline to 502 and 1,627 units/L, respectively, at 4 hours postinfusion. These indexes returned to normal within 24 hours after infusion. This patient was not given ING-1 again.

No other DLT was observed, with no DLT documented outside course one and no evidence of cumulative toxicity.

Pharmacokinetics

All 25 patients had plasma sampled in the first course for pharmacokinetic studies. Complete sampling was done in all of the patients. ING-1 concentration data were well fit by a one-compartment model. Fig. 1 shows the average concentrations of ING-1, administered on a 3-week schedule, plotted against time for the first 14 days. As noted in the methods section, because CL seemed to decrease with increasing dosage (Fig. 2), the pharmacokinetic data were fitted with both constant CL and dose-varying CL models. On the basis of a χ² test on the goodness-of-fit statistics of the two models (−2 times the negative log likelihood, at 1 df or −2LL), the dose-varying CL model resulted in an improved fit (P < 0.0001). This suggested that CL decreased with increasing dosage level, perhaps because of saturable antigen binding. To determine whether a second phase could be distinguished from the data, the data were also fitted with a two-compartment model with the dose-varying CL model. The goodness of fit (−2LL) of this resulting model was −400.782, which differed from the one-compartment model by 12.619 units. The χ² statistic was not statistically significant (P = 0.181, 9 df, when taking into account additional population parameters and variances required for the two-compartment model), which suggests that a two-compartment model fit was not justified for the data.

The pharmacokinetic parameters for patients treated at each dose level are presented in Table 4. The parameters of the patients who experienced DLT did not differ from those of
patients who did not suffer DLT. Plasma ING-1 pharmacokinetic parameters with the once-a-week schedule were not significantly different from those observed with the once-every-3 weeks schedule (Table 4). Fig. 2 depicts the relationships between these variables and dose. ING-1 concentrations declined with a half-life (mean ± SEM) of 14.0 ± 2.4, 20.6 ± 2.2, 31.4 ± 2.1, and 37.8 ± 3.2 h at 0.03, 0.1, 0.3, and 1.0 mg/kg, respectively. These half-lives are much shorter than the 14-day half-life observed in cynomolgus monkeys (33). Regardless of dose, the Vc of ING-1 ranged between 47.8 and 50.1 mL/kg, with a mean (±SEM) of 46.6 ± 1.6 mL/kg which is similar to plasma volume. Linear regression analysis of Vc (in liters) versus body weight (Fig. 3) indicates a trend of increasing Vc with increasing body weight (r² = 0.531). Linear regression of CL in liters per day versus body weight are also shown in Fig. 3 and reveal little evidence of any relationship between these two parameters (r² = 0.02). Therefore, although dosage based on body weight may decrease interindividual variability in Cmax, it may not decrease interindividual variability in area under the curve (AUC).

Biodistribution

The percentage of injection dose (%ID) versus time for the whole body, selected tissues, and the liver metastases are presented in Fig. 4. The whole body half-life of radiolabeled ING-1 ranged from 72.4 to 87.7 hours, which is substantially longer than the plasma half-life of ING-1 at the same dosage level (20.6 ± 2.2 hours). Tumors and kidneys had relatively long retention times when compared with other organs and the whole body (Table 5). No thyroid uptake was documented. Pancreatic uptake could not be specifically determined. Selected images from the three patients treated with radiolabeled ING-1 are presented in Fig. 5. The images obtained from the first patient, who had metastatic pancreatic cancer, revealed a necrotic liver metastasis (Fig. 5A). The images acquired from a second patient, who had metastatic prostate cancer, showed abnormal uptake in a metastatic deposit in a right external iliac node (Fig. 5B). The third patient, who had colorectal cancer, had substantial localization of 131I-labeled ING-1 in two liver metastases (Fig. 5C).

![Fig. 1](https://cancercreses.aacrjournals.org/)

**Fig. 1** Mean plasma concentration-time profiles of ING-1 in course one for each dose level.
because of the diffuse distribution of hepatic metastases (Fig. 4F and G).

**Human Antihuman Antibody Response**

Twenty of 25 patients had samples that were evaluable for human antihuman antibody/antibodies (HAHA) response. No antibodies to ING-1 were detected in the pretreatment samples. HAHA were detected in 2 (11.8%) of 20 patients, with HAHA detected in 2 patients treated at 0.3 mg/kg. One patient had a HAHA of 11 ng/mL after the second dose, which increased to 188 ng/mL after a subsequent dose. The second patient developed a HAHA of 31 ng/mL after the third dose. Competition experiments suggest that these antibody responses were directed toward the variable region. The pharmacokinetics of ING-1 during repeated dosing was not significantly altered in these two patients.

**Antitumor Activity**

There were no objective responses. A patient treated at 0.03 mg/kg had stable disease at 12 weeks and a decrement in carcinoembryonic antigen from 6,600 ng/mL to 1,394 ng/mL at day 43, which increased to 6,582 ng/mL by day 84. This patient...
had radiologic evidence of progressive disease after 18 weeks of treatment. Another patient with colorectal cancer had stable disease at 12 weeks, but progressive disease was documented after 20 weeks of therapy.

**DISCUSSION**

The high prevalence of abnormally high levels of Ep-CAM in human epithelial carcinomas and the evidence for the role of Ep-CAM in the modulation of tumor cell differentiation and proliferation through the cellular cytoskeleton, make this antigen a promising target for antitumor therapy (16, 17). Ep-CAM may be an important target for the treatment of patients with breast cancer, in which its overexpression determined by immunohistochemistry strongly correlates with histologic grading and predicts survival independent of tumor size, histologic grade, nodal status, or hormone receptor expression in metastatic breast cancer (10, 12–14). The finding that Ep-CAM expressed on normal epithelium is not as accessible to therapeutic targeting as Ep-CAM expressed on tumor cells suggests that selective cytotoxicity may be achievable when targeting Ep-CAM by monoclonal antibody therapy, allowing a favorable therapeutic index (18). ING-1 is a Human-Engineered monoclonal antibody (31, 32) to Ep-CAM that may have a superior clinical antitumor profile to edrecolomab because of its higher antigen affinity and the abrogation of the HAMA response (20, 39).

![Graphs](https://example.com/graphs.png)

**Fig. 3** Scatterplots depicting the relationship of ING-1 CL and Vc values as a function of subject body weight; fit of the data derived from linear least squares regression.

### Table 4. Compartmental pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Dose Form</th>
<th>AUC µg/mL-h (per dose)</th>
<th>Cmax µg/mL</th>
<th>Vc mL/kg</th>
<th>CL mL/kg/d</th>
<th>t1/2 α hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03 mg/kg (n = 4)</td>
<td>12.7 ± 3.9</td>
<td>0.63 ± 0.09</td>
<td>47.8 ± 6.6</td>
<td>56.7 ± 17.3</td>
<td>14.0 ± 2.4</td>
</tr>
<tr>
<td>0.1 mg/kg (n = 4)</td>
<td>55.3 ± 13.1</td>
<td>2.00 ± 0.22</td>
<td>50.1 ± 5.4</td>
<td>43.4 ± 10.3</td>
<td>19.2 ± 2.5</td>
</tr>
<tr>
<td>0.1 mg/kg weekly (n = 3)</td>
<td>68.4 ± 21.7</td>
<td>2.09 ± 0.28</td>
<td>47.9 ± 6.4</td>
<td>35.1 ± 11.1</td>
<td>22.7 ± 4.3</td>
</tr>
<tr>
<td>0.3 mg/kg (n = 12)</td>
<td>307 ± 30</td>
<td>6.78 ± 0.25</td>
<td>44.3 ± 1.6</td>
<td>23.4 ± 2.3</td>
<td>31.4 ± 2.1</td>
</tr>
<tr>
<td>1.0 mg/kg (n = 2)</td>
<td>1103 ± 102</td>
<td>20.3 ± 0.2</td>
<td>49.4 ± 0.4</td>
<td>21.8 ± 2.0</td>
<td>37.8 ± 3.2</td>
</tr>
</tbody>
</table>

| Patient 1 | 1210 | 20.4 | 48.9 | 19.8 | 41.1 |
| Patient 2 | 1005 | 20.1 | 49.8 | 23.9 | 34.7 |

**NOTE.** Values are mean ± SEM. For 1.0 mg/kg dose, only two patients were treated, therefore SEM could not be estimated.

**Abbreviations:** AUC, area under the curve; Cmax, peak concentration (compartmental); Vc, volume of distribution of the central compartment; CL, clearance; \( t_{1/2,\alpha} = \alpha \text{ half-life} \).
Fig. 4 Remaining radioactivity determined by a gamma detector as a function of time in whole body (A), spleen (B), bladder (C), kidney (D), lungs (E), liver (F), and tumor (G). Values are for the pancreatic cancer (■), prostate cancer (▲), and colorectal cancer patients (●). In G, the two curves represent the two liver metastases in the patient with colorectal cancer.
malaise, dizziness, fatigue, nausea and/or vomiting, abdominal cramps, and unspecified cardiovascular effects (27). These toxicities were uncommon with ING-1. HAHA responses to ING-1 were detectable in two patients (11.8%), which is significantly less than the 80% HAMA response observed with edrecolomab, and comparable with other humanized monoclonal antibodies (28, 40, 41).

Pancreatitis was the DLT of ING-1 and was observed in one patient at 1.0 mg/kg, with grade 3 asymptomatic amylase elevation also observed at 0.3 mg/kg. No evidence of pancreatitis was observed in seven patients given a dose of 0.1 mg/kg. Previous studies with the murine monoclonal antibody CO17-1A have described Ep-CAM expression in normal pancreatic tissue (42). Moreover, clinical studies with the high-affinity humanized monoclonal antibody, 3622W94 (Glaxo-Smith-Kline), have also described transient and asymptomatic increases in pancreatic enzymes at lower doses and one case of pancreatitis (43, 44). These data suggest that antibodies with higher affinity to Ep-CAM are not only more cytotoxic to Ep-CAM-positive tumor cells but can also induce rapid pancreatic toxic injury and amylase release. Although it is envisioned that the higher expression of Ep-CAM in tumor cells and the low accessibility of Ep-CAM in normal cells may allow a therapeutic window for ING-1 (17, 18), these clinical data raise concern that high-affinity antibodies to Ep-CAM may have limited selectivity for tumor cells. Moreover, future studies with such antibodies should exclude patients who have a history of pancreatitis or alcohol abuse and, perhaps, those who have tumors associated with biliary obstruction, and should assess amylase and lipase levels during screening.

The pharmacokinetic behavior of ING-1 was predicted to

Table 5  Tissue half-life of radioactivity

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreatic cancer</td>
<td>Prostate cancer</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Whole body</td>
<td>78.1</td>
<td>87.7</td>
<td>72.4</td>
</tr>
<tr>
<td>Liver</td>
<td>55.5</td>
<td>50.8</td>
<td>96.8*</td>
</tr>
<tr>
<td>Spleen</td>
<td>45.4</td>
<td>NA</td>
<td>23.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>180.8</td>
<td>103</td>
<td>245.7</td>
</tr>
<tr>
<td>Lungs</td>
<td>59.3</td>
<td>43.1</td>
<td>51.9</td>
</tr>
<tr>
<td>Tumor 1</td>
<td>NA</td>
<td>NA</td>
<td>155.3</td>
</tr>
<tr>
<td>Tumor 2</td>
<td>NA</td>
<td>NA</td>
<td>96.7</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.
* Patient 3 had extensive liver metastases.

Fig. 5 Imaging of radiolabeled ING-1 distribution. A, patient 1 with pancreatic cancer depicting necrotic tumor in the liver. B, patient 2 with prostate cancer depicting a right external iliac nodal metastases. C, patient 3 with colorectal cancer depicting two liver metastases.
be similar to that of human IgG (45, 46). Based on this information, and the data obtained in cynomolgus monkeys, (33) a 0.1-mg/kg dose on a once-every-3-weeks schedule was projected to maintain a plasma concentration above the biologically relevant concentration of 0.1 μg/mL for 21 days (31). However, the plasma half-life of ING-1 in this clinical trial was considerably shorter than predicted and was shorter than that reported with other therapeutic antibodies. This may have been the result of the higher affinity of the antibody for human Ep-CAM. An alternative explanation is inadequate humanization of the antibody in view of the observed HAHA responses, but this appears unlikely in view of the primate pharmacokinetic data which demonstrated a half-life of 14 days, although it cannot be ruled out (33). The studies performed with radiolabeled ING-1 also suggest some degree of antibody localization to normal tissues, which with tumor tissue may have provided an Ep-CAM “sink” resulting in a shorter half-life. This is also supported by the longer half-lives observed with repeated dosing and may help explain the observed toxicities. Evidence for the possible binding of ING-1 to normal human tissue is supported by immunohistochemical evaluation of ING-1 cross-reactivity with normal human tissues. Epithelial cell surface staining was reported along the basolateral surface and ranged from moderate to intense, localizing to the epithelia of most gastrointestinal and genitourinary tract mucosa including the pancreas and prostate and the bronchiolar epithelium of the lung. Staining was also observed on selected mesothelial cells of the ovary (one of one), small intestine (two of two), and large intestine (one of two).

Because of the shortened half-life, ING-1 plasma concentrations were above 0.1 μg/mL for only 4 days in patients who received dosage at 0.1 mg/kg. This suggested that to maintain a biologically relevant ING-1 concentration, the antibody should be administered more frequently. More frequent administration also lowers peak levels of ING-1, maintaining dosage intensity. A once-a-week schedule was, therefore, studied. No evidence of pancreatitis or other DLT was observed in seven patients who were given 0.1 mg/kg of ING-1 once a week; and 0.1 mg/kg administered once a week was the dose recommended for future efficacy trials.

The whole body half-life of radiolabeled 131I-labeled ING-1 was approximately three times longer than the plasma half-life, which suggests that ING-1 plasma pharmacokinetics may overestimate ING-1 clearance. Hepatic uptake of radiolabeled ING-1 was observed in keeping with previous studies with other monoclonal antibodies that have demonstrated that the liver can take up to 15% of the administered dose of radiolabeled antibody (47). It has been suggested that this is due to extravascular pooling of antibody and not antibody–antigen interactions, which is in keeping with the absence of induced transaminitis or hepatic toxicity in this clinical trial. Increased uptake to hepatic and nodal metastatic deposits, from colorectal and prostate carcinomas, respectively, was also observed with radioactivity present in tumor tissue after the end of the first week, indicating sustained delivery of ING-1 to tumor.

To conclude, biologically relevant plasma levels of ING-1 may be achieved in patients with advanced adenocarcinomas at doses that are well tolerated. In addition, we have shown that 131I-labeled ING-1 localized to tumor and has a tissue half-life that is longer than the plasma half-life.

REFERENCES


ING-1, a Monoclonal Antibody Targeting Ep-CAM in Patients with Advanced Adenocarcinomas

Johann S. de Bono, Anthony W. Tolcher, Andre Forero, et al.