Factors Influencing the Pharmacokinetics, Dosimetry, and Diagnostic Accuracy of Radioimmunodetection and Radioimmunotherapy of Carcinoembryonic Antigen-expressing Tumors

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ABSTRACT

The aim of this study was to examine factors that may influence the pharmacokinetics, diagnostic accuracy, and dosimetry in radioimmunodetection and radioimmunotherapy with anti-carcinoembryonic antigen (CEA) monoclonal antibodies (mAbs). Data from 275 patients with CEA-expressing tumors were analyzed retrospectively. Of these, 69 patients devoid of human antimouse antibody (i.e., 31 colorectal, 9 lung, 7 breast, 4 ovarian, 6 pancreatic, 9 medullary thyroid, 1 gallbladder, and 1 salivary gland cancer, and 1 primary tumor of unknown origin) underwent a low-protein-dose diagnostic study (0.3–2.6 mg of protein; 6.8–28.8 mCi 131I-labeled IgG or fragments), followed within 4 weeks by a high-protein-dose therapy injection (4.0–275 mg of protein; 29.8–2389 mCi). The anti-CEA antibodies NP-4 (Kd = 104 M−1) and MN-14 (Kd = 106 M−1) were used. Plasma clearance, the molecular composition of radioactivity in the plasma, and the cumulated activity in organs and tumors were determined. Radiation doses were derived from the Medical Internal Radiation Dose scheme. At a low-protein dose and over a similar range of plasma CEA, a significantly higher percentage of MN-14 than of NP-4 was complexed with circulating CEA, consistent with its higher affinity. Complexation was reduced with increasing protein doses. However, the targeting sensitivity was not affected. Profound differences were found in the clearance of the antibody between different types of cancer. Colorectal cancer patients cleared the antibody significantly faster from blood (T1/2 = 17.6 ± 12.6 versus 44.2 ± 23.7 h) and whole body (T1/2 = 53.2 ± 30.1 versus 114.6 ± 59.7 h) than other tumor types (P < 0.001). Consequently, significantly lower red marrow (2.1 ± 1.0 cGy/mCi versus 4.3 ± 1.6 cGy/mCi) and whole-body doses (0.5 ± 0.3 cGy/mCi versus 1.0 ± 0.4 cGy/mCi) were seen in colorectal cancer patients as compared with other tumor types (P < 0.001). This clearance is probably due to hepatic metabolism of the immune complexes. Clearance rates were especially high in patients with colorectal cancer having large liver metastases and elevated liver enzymes (rapid hepatic clearance with liberation of free 131I). In contrast, a disease-stage and plasma CEA-matched cohort of colorectal cancer patients, examined with the 131I-labeled anti-colon-specific antigen p mAb Mu-9, showed normal murine IgG pharmacokinetics (n = 22; 3 of them compared intrindividually to MN-14). Only in colorectal cancer patients did complexes between mAb and CEA tend to clear rapidly, whereas Mu-9 had normal kinetics in these patients. This suggests that different CEA-expressing cancer types may produce heterogeneous CEA molecules and that the variability in mAb clearance is due to varying clearance rates of these different circulating CEA subspecies. Disease-related alterations in antibody metabolism are unlikely, given that only anti-CEA antibodies exhibit this phenomenon.

INTRODUCTION

Since the initial work of Goldenberg et al. (1), who successfully used affinity-purified, polyclonal 131I-labeled antisera in the RAID3 of CEA-expressing tumors for the first time, and with the development of the hybridoma technique by Köhler and Milstein (2), numerous antibodies directed against a variety of different tumor antigens have been used in RAID and RAIT trials. In non-Hodgkin’s lymphoma, RAIT is advancing to become a third mode of therapy, alongside chemotherapy and external beam radiation (3–5). In solid tumors, therapeutic success is still limited, but ongoing trials are promising, especially in small-volume disease (6)4 and medullary thyroid (7, 8) and ovarian carcinomas (9).

There are several factors that potentially may affect antibody pharmacokinetics and tumor targeting. For example, in the case of most lymphoma mAbs, the protein dose plays a critical role in influencing their biodistribution (3, 4). In this case, the easily accessible excess of antigen in normal tissue (e.g., spleen and other lymphatic tissue) may act as an antigenic sink, preventing successful tumor targeting. For antigens that have only a low level of expression in normal tissues or are less accessible there, as is the case for most oncofetal antigens, these considerations should be less critical. However, circulating antigen shed by the tumor cells may interact with the injected antibody, thus influencing its pharmacokinetics (10). There have been some clinical observations of altered antibody clearance in patients with highly elevated circulating tumor marker levels (11, 12). A large variety of both preclinical and clinical studies have attempted to correlate the administered protein dose of anti-CEA or anti-melanoma antibodies with the diagnostic accuracy and dosimetry of these antibodies (13–26). Clinically, these comparisons usually were performed interindividually. Generally, no profound differences in tumor targeting or dosimetry were observed (10, 16, 25), although some authors found increasing imaging sensitivities with increasing amounts of protein (14, 17–22). These data were reviewed recently by Pimm (10), who concluded that circulating antigen does not significantly affect tumor targeting in patients, whereas in animals, profound alterations of mAb pharmacokinetics occur. There have been several studies addressing the influence of HAMA on the biodistribution and targeting of antibodies (6, 27–30), but to the best of our knowledge, no study has been published analyzing in detail other possible factors that might influence the pharmacoki-
netics, diagnostic accuracy, and dosimetry of radiolabeled anti-CEA antibodies in patients. Therefore, the aim of our study was to analyze, retrospectively, which factors may influence these parameters in patients with different CEA-expressing tumors. Additionally, we performed an intraindividual analysis of the effect of the applied antibody protein dose in the same patient, which may allow more detailed observations in a lower number of individuals than comparisons of different cohorts.

MATERIALS AND METHODS

Antibodies and Radiolabeling. NP-4 (IMMU-4; Immunomedics, Inc., Morris Plains, NJ; \( K_a = 10^8 \text{l/mol} \)) and MN-14 (IMMU-14; Immunomedics, Inc.; \( K_a = 10^8 \text{l/mol} \)) are IgG1 murine mAbs directed against CEA. Preclinical and clinical experiences with these antibodies were described previously (6—8, 24, 25, 31—34). Their final purity was tested by immunoelectrophoresis, polyacrylamide gel, and size-exclusion HPLC. The F(ab')2 and F(ab)2 fragments of NP-4 and MN-14, respectively, were prepared by pepsin and papain digestion.

Radioiodination with Na131I was performed by the chloramine-T or Iodo-Gen methods (39). The specific activity was 12—16 mCi/mg. Binding of the radiiodinated antibodies to a CEA-immunoadsorbent column was more than 80%. In labeled fragments, more than 85% was in the form of \( M_f 100,000 \) compounds, with less than 15% in the form of \( M_f 50,000 \) fragments (Fab' or Fab). \(^{131} \text{I}-\text{Mu-9} \) labeling had an immunoreactivity of 74.2 ± 7.5% (37).

CMA and HAMA Determinations. Plasma CMA levels were determined by using a CEA enzyme-linked immunoassay, which has been described elsewhere (40). HAMA titers were determined, as described previously, as titers, or as a commercially available ELISA (ImmuSTRIP HAMA-EIA; Immunomedics, Inc.; Ref. 41). The normal range in these assays was a titer less than 100, which corresponds to a concentration of less than 74 ng/ml.

Patient Selection. A total of 275 patients underwent RAID and/or RAIT studies with the \(^{131} \text{I}-\text{anti-CEA} \) mAbs NP-4 or MN-14 (including hMN-14) in our institution until May 1995. Of these, 69 patients [NP-4: \( n = 30, 29 \) of them IgG, 1 F(ab')2; MN-14: \( n = 39, 32 \) of them IgG (7 humanized IgG), 7 F(ab')2] among the 69 patients were 31 colorectal, 9 lung, 7 breast, 4 ovarian, 6 pancreatic, 9 medullary thyroid, 1 gallbladder, and 1 salivary gland cancer, as well as 1 CEA-expressing tumor of unknown origin.

Table 1 Overview of the 69 HAMA-negative patients who underwent two RAID/RAIT studies with different antibody protein doses within 4 weeks

<table>
<thead>
<tr>
<th>Cancer type (no. patients)</th>
<th>Age (yr) (male/female)</th>
<th>mAb</th>
<th>Plasma CEA range (ng/ml)</th>
<th>Diagnostic</th>
<th>Therapeutic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg</td>
<td>mCi</td>
</tr>
<tr>
<td>Colorectal 13</td>
<td>44—74 (9/4)</td>
<td>NP-4</td>
<td>30—3617</td>
<td>0.3—2.0</td>
<td>6.8—25.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 IgG</td>
<td></td>
<td>0.7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 F(ab')2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MN-14</td>
<td>2.6—21644</td>
<td>0.3—2.6</td>
<td>8.0—30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 IgG</td>
<td></td>
<td>170</td>
<td>7.6</td>
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<tr>
<td></td>
<td></td>
<td>5 hlgG</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1 F(ab')2</td>
<td></td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Medullary thyroid 9</td>
<td>15—76 (7/2)</td>
<td>MN-14</td>
<td>11—3363</td>
<td>0.6—1.0</td>
<td>8.1—8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 IgG</td>
<td></td>
<td>0.6—1.0</td>
<td>8.0—8.4</td>
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<tr>
<td></td>
<td></td>
<td>6 F(ab')2</td>
<td></td>
<td>7.9—15</td>
<td>90—148</td>
</tr>
<tr>
<td>Lung 6</td>
<td>48—69 (3/3)</td>
<td>NP-4</td>
<td>0.3—547</td>
<td>0.6—2.3</td>
<td>5.0—27.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 IgG</td>
<td></td>
<td>0.6—0.8</td>
<td>8.0—8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MN-14</td>
<td>2.6—135</td>
<td>0.7—1.9</td>
<td>9.4—26</td>
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<tr>
<td>Pancreatic 4</td>
<td>58—78 (3/1)</td>
<td>NP-4</td>
<td>2.1—120</td>
<td>1.0—1.1</td>
<td>9.5—15</td>
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<td></td>
<td>1 IgG</td>
<td></td>
<td>2.6—79</td>
<td>6.8—8.5</td>
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<td></td>
<td></td>
<td>1 F(ab')2</td>
<td></td>
<td>91—121</td>
<td></td>
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<tr>
<td>Ovarian 4</td>
<td>39—71 (0/4)</td>
<td>MN-14</td>
<td>2.6—14</td>
<td>0.6</td>
<td>8.0—8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 IgG</td>
<td></td>
<td>4.2—6.2</td>
<td>59—81</td>
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<td></td>
<td>1 hlgG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast 5</td>
<td>27—57 (0/5)</td>
<td>NP-4</td>
<td>1.6—1968</td>
<td>0.7—1.7</td>
<td>9.7—25</td>
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<tr>
<td></td>
<td></td>
<td>3 IgG</td>
<td></td>
<td>0.6—2.4</td>
<td>8.1—29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 MN-14</td>
<td></td>
<td>6.8—20</td>
<td>30—85</td>
</tr>
<tr>
<td>Other(^a)</td>
<td>45—51 (2/0)</td>
<td>NP-4</td>
<td>3.2</td>
<td>0.9—1.4</td>
<td>9.2—24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 MN-14</td>
<td></td>
<td>5.7—15</td>
<td>98—210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hlgG</td>
<td></td>
<td>10.1</td>
<td>148</td>
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</tbody>
</table>

\(^a\) One biliary, one salivary gland, and one CEA-expressing cancer of unknown origin.
Pharmacokinetic Analysis. Blood clearance rates were determined by counting samples of whole blood at various times after the end of the infusion. Mostly, blood samples were collected at 5 and 30 min and 1, 2, 4, 8, 24, 48, 72, and 96 h after the end of the antibody infusion until the end of imaging. Clearance rates were calculated for each patient using the least-squares analysis as described previously (24, 34, 35). The blood clearance was expressed as either a single or a biexponential function, based on the best fit. The half-life ($T_{1/2}$) was defined as the number of hours required for 50% of the activity to be removed from the blood.

Total-body clearance rates were determined from whole-body scans obtained until the end of the imaging time or, for patients receiving more than 30 mCi of activity, by a hand-held rate meter with measurements initiated immediately at the end of infusion, followed by twice-daily readings until the end of the study. The total-body activity was expressed as a linear rate of clearance.

Analysis of the Molecular Composition of Plasma Samples. Plasma samples taken 1 and 24 h after antibody administration were analyzed by size-exclusion HPLC (Bio-Sil SEC-250 column, 300 × 7.8 mm; Bio-Rad Laboratories, Richmond, CA). Native radiolabeled antibodies and their fragments, as well as iodide and moniodotyrosine, were run as standards. Molar ratios between plasma CEA and the injected antibody protein were calculated based on the ng/ml of plasma CEA and by dividing the total antibody protein injected by the estimated plasma volume of the patients to derive the ng/ml of the antibody in the blood at time 0.

Dosimetry. The amount of radioactivity in the organs and tumors was determined from regions of interest generated from the anterior and posterior planar views that were obtained during each imaging session. Appropriate adjacent soft-tissue regions served as background reference regions for the organs. The background regions chosen for tumors were the normal non-tumor-bearing parenchyma of the respective organ, as close to the tumor as possible. The activities in these regions were generated using the build-up factor methodology (42). The individual time-activity curves of organs and tumors were fit to a mono- or biexponential function using a nonlinear least-squares analysis or the trapezoidal method and then integrated to obtain the cumulated activity in each region (43-45). The blood time-activity concentration data were also fit by an exponential function (see above) to obtain the cumulated activity in the blood. The red marrow-cumulated activity was calculated from the blood and whole-body data by multiplying this concentration by 1500, as the assumed weight in grams of the marrow in an average adult. The mean dose was calculated for organs and tumors according to the Medical Internal Radiation Dose scheme, with correction for the remainder of the whole-body activity (43-45). The masses of normal organs were generated from Medical Internal Radiation Dose standard tables (46), and tumor volumes were obtained from computed tomography or single-photon emission computed tomography data, as described earlier (6).

Statistical Analysis. All values reported herein represent the arithmetic means with the corresponding SDs. Statistical analysis of two-sample comparisons was performed with the Student’s $t$ test. For analysis of paired observations, the nonparametric sign test was used (47). The relationship of the first and second components of the pair was studied by fitting a linear regression model. Linear regression lines were fit using the weighted least-squares method. By estimating SEs, prediction intervals were constructed. SDs were estimated via residuals, and pointwise 95% confidence intervals were constructed using normal distribution tables. Data analysis and statistical computing were based on the statistical software S (48).

RESULTS

Plasma CEA, Complexation, and Metabolic Fate of These Complexes in Relation to Different Tumor Types. Table 1 shows an overview of 69 of 275 patients who underwent two studies with the anti-CEA mAbs NP-4 or MN-14, fulfilling the assessment criteria. Colorectal cancer patients had the highest plasma CEA levels (range, 2.6–21,644 ng/ml; median, 271 ng/ml); overall, the range for non-colorectal cancer patients was 0.3–1967 ng/ml, with a median of 50.3 ng/ml). Within the same type of cancer, similar levels of CEA were observed between patients examined with NP-4 or MN-14.

Consistent with its 10-fold higher affinity, at comparable CEA levels, MN-14 complexed more with plasma CEA than NP-4 (Fig. 1). The percentage of complexes formed was characterized by a sigmoid-shaped
curve in relation to the plasma CEA. Fig. 1 shows these data expressed as molar ratios of CEA:mAb in the blood (Fig. 1: top, low-protein diagnostic study; bottom, high-protein therapeutic injection). Fifty % of the MN-14 was complexed at a molar ratio of approximately 1:1, and above ratios of 5:1, complexation was virtually 100% (Fig. 1, top). At higher protein doses (i.e., lowering the CEA:mAb ratio), only four patients continued to show marked complexation (Fig. 1, bottom). Fragments of NP-4 and MN-14 behaved similarly to the IgG, and there was no difference seen between the murine form of MN-14 and its complementarity-determining region-grafted, humanized form, as has already been shown earlier (35). Because the behavior of the murine and humanized forms of MN-14 was identical, both will not be differentiated further, unless otherwise stated.

A similar sigmoid-shaped curve was seen when plotting the percentage complexation data against the plasma CEA levels. At low-protein doses and at CEA levels less than 100 ng/ml, complexation was usually less than 15% for MN-14 and less than 5% for NP-4. With MN-14, approximately 50% complexation was observed at a plasma CEA of 250 ng/ml; at CEA levels greater than 1100 ng/ml, more than 90% of the injected antibody was complexed.

A fundamental difference was observed with respect to the metabolic fate of these complexes. At comparable CEA levels, only colorectal cancer patients tended to produce substantial amounts of low-molecular-weight substances in the 24-h plasma chromatography profiles (data not shown). No such metabolic breakdown was seen in all other forms of CEA-expressing tumors, independent of the plasma CEA or complexation. Occasionally, in some colorectal cancer patients, low amounts of complexation at 1 h p.i. were associated with substantial amounts of metabolites in the 24-h plasma. This may represent an especially rapid metabolism of these complexes.

Blood and Whole-Body Half-Lives of MN-14 and NP-4 Depend on the Type of Cancer and the Protein Dose. When examining the blood clearance of both monoclonal anti-CEA antibodies, it was apparent that colorectal cancer patients, as a group, had cleared the mAbs from the blood more quickly than patients with other types of cancer (Fig. 2). At low-protein doses, colorectal cancer patients
showed significantly lower blood half-lives than patients with all other types of cancer (blood $T_{1/2}$: 15.8 ± 12.6 versus 46.2 ± 29.5 h in colorectal versus other tumor types for MN-14 and 19.9 ± 10.6 h versus 42.3 ± 12.2 h for NP-4; $P < 0.001$). With MN-14, this difference seemed to be slightly more pronounced than with NP-4. This result is consistent with its 10-fold higher affinity but is not statistically significant. Because of the similarity in biokinetics between MN-14 and NP-4, they will not be discussed separately in the following analyses, unless stated otherwise. The overall blood $T_{1/2}$ (i.e., NP-4 and MN-14 together) in colorectal cancer was 17.6 ± 12.6 h ($n = 29$) versus 44.2 ± 23.7 h in all other tumor types ($n = 32$; $P < 0.001$).

When the protein dose was increased, these blood half-lives showed a tendency to increase in colorectal cancer patients given MN-14 (Fig. 2). This trend was significant at $P = 0.017$, but the mean clearance times were not significantly different. Similar observations in the blood $T_{1/2}$s were made for whole-body half-lives (Fig. 2). For both mAbs, the blood $T_{1/2}$s were 53.2 ± 30.1 h in colorectal cancer versus 114.6 ± 59.7 h in other tumor types ($P < 0.001$), which again shows a significant tendency to increase the blood half-life with higher protein doses ($P = 0.034$).

Fig. 3 shows the blood half-lives in relation to the tumor types and plasma CEA levels. Patients with normal plasma CEA (less than 5 ng/ml) had blood $T_{1/2}$s of more than 20 h, regardless of the tumor type. At plasma CEA levels of more than 10 ng/ml, the blood half-lives of colorectal cancer patients became significantly lower than in all other types of cancer at these elevated levels ($P < 0.004$ for the low-protein study (Fig. 3, top); $P < 0.002$ for the high-protein study (Fig. 3, bottom)). With increasing plasma CEA, these $T_{1/2}$s showed a decreasing tendency. A linear regression line shows, in colorectal cancer patients, decreasing blood half-lives with increasing plasma CEA (Fig. 3, dashed lines; slope different from 0 at $P = 0.0003$). However, in an individual case, half-lives were difficult to predict from the amounts of CEA in the blood. This phenomenon is in agreement with the rapid metabolic processing of the antibody-CEA complexes, which was found only in colorectal cancer patients, where such rapid clearance was already observed at CEA levels as low as 30 ng/ml, both with NP-4 and MN-14 (Fig. 3).

Especially short half-lives were observed in colorectal cancer patients having liver metastases, and typically, blood $T_{1/2}$s were the shortest the higher the tumor burden (data not shown). Five colorectal cancer patients without liver metastases (Fig. 3, stars) had, at similar CEA levels, significantly longer half-lives ($P < 0.05$) than those with liver involvement (Fig. 3). In the latter, an unusually high uptake in early (4 h) scans was noticed in the remaining normal liver parenchyma (Fig. 4). This suggests that complexes of CEA and the antibody were cleared rapidly via the liver, with subsequent fast catabolism and liberation of substances in the low-molecular-weight range (partially free iodide, as indicated by its increased uptake in the stomach and the thyroid, starting generally in the 24-h scans; Fig. 4).

The plasma HPLC profiles of this subset of colorectal cancer patients were characterized by substantial amounts of complexation at 1 h p.i. and by high amounts of low-molecular-weight compounds (usually as a double peak) at 24 h p.i. Although blood half-lives showed a statistically significant tendency to increase in these patients when the protein dose was increased ($P < 0.02$), at the highest protein doses tested, these half-lives remained substantially shorter than the blood half-lives found in other cancer types (Figs. 2–4). In contrast to a monoexponential behavior in patients with blood half-lives longer than 20–25 h (Fig. 4c, right), the organ kinetics typically showed a biexponential clearance type. Because of these extremely short half-lives, maximum tumor uptake was confined to the first few hours after antibody administration (compare Fig. 4c, left).

An interesting phenomenon occurred in all three colorectal cancer patients who had plasma CEA in excess of 3000 ng/ml. In these patients, the plasma half-lives of NP-4, MN-14, or hMN-14 were actually higher than expected, based on the trend for more accelerated clearance as plasma CEA levels increased. For example, patient 1576 had more than 70% of her liver replaced by tumor and a plasma CEA of 21,643 ng/ml. The blood half-life of 1.5 mg hMN-14 IgG, at 25.7 h, and the whole-body $t_{1/2}$, at 171.2 h, were relatively long, compared with the values expected from extrapolating the data from lower plasma CEA levels. The reason for this phenomenon is probably a competitive blocking of the CEA receptors in the liver by this exceedingly high plasma CEA.

All rapidly clearing patients had elevated liver cholestasis values (AP) and often also biochemical evidence of liver parenchymal damage (elevated AST), whereas in colorectal cancer, elevated liver dysfunction parameters (especially AP) correlated with especially rapid plasma and whole-body clearance rates (Fig. 5a). No such trend was seen in patients with other types of cancer over a range of comparable AP and AST levels (Fig. 5b).

Especially long half-lives were observed in some lung and MTC patients. An illustration is patient 1547, who was a 15-year-old boy with familial multiple endocrine neoplasia (MEN 2b) syndrome and widespread metastatic MTC (Fig. 6). His plasma CEA on his first injection of MN-14 F(ab)$_2$ fragment (0.8 mg) was 299 ng/ml. HPLC of the plasma 1 h p.i. showed 89.5% of activity in the high-molecular-weight fraction. These complexes cleared out of the blood extremely slowly (blood $T_{1/2}$, 46.9 h; whole-body $t_{1/2}$, 60.3 h). With about a 10-fold higher protein dose (10 mg) in the
**Fig. 4.** Patient 1516, a 72-year-old man (plasma CEA level, 54.6 ng/ml) with large liver metastases of a rectosigmoid cancer. 

**a.** Targeting sequence of the diagnostic injection (0.7 mg; 8.4 mCi 131I-labeled MN-14 IgG) with rapid hepatic clearance of the antibody (enhanced liver uptake at 4 h p.i.) and liberation of free iodide (thyroid and stomach uptake). Blood $T^{1/2}$ = 5.2 h; whole-body $T_{1/2}$ = 30.7 h. 

**b.** Comparison of the diagnostic study and the 10-fold higher therapeutic dose (9.9 mg; 106.7 mCi) in the same patient shown at 48 h p.i. The amount of metabolites appears substantially lower, and the blood and whole-body half-lives longer, although still substantially lower than average. Blood $T^{1/2}$ = 10.5 h; whole-body $T_{1/2}$ = 37.6 h. 

**c.** Targeting kinetics of this patient with a biexponential rapid clearance of the antibody from the organs and tumors (left) and, for comparison, the monoeXponential kinetics in a slowly clearing colorectal cancer patient (solitary lung metastases, plasma CEA 2.3 ng/ml; right). Filled symbols, low-protein studies; open symbols, high-protein (therapeutic) studies.

therapeutic study, complexation dropped to 15.6%, with a subsequent blood-$T^{1/2}$ of 21.9 h.

Although only 8 patients studied with bivalent fragments were included in this study, the tendency of these fragments toward shorter half-lives in colorectal cancer patients was less than that seen in patients given IgG (blood $T^{1/2}$ 17.4 and 13.4 at CEA levels of 80.6 and 170 ng/ml, respectively; $n = 2$). However, prolonged half-lives in MTC patients were more pronounced with fragments than with IgG (31.3 ± 15.9 h, with a range from 13.5 to 61.7 h at low-protein doses and a CEA range between 5.0 and 652 ng/ml; $n = 6$). The "normal" blood $T^{1/2}$ of MN-14 F(ab)$_2$ at low plasma CEA levels was reported by Juweid et al. (49) as 16.8 ± 4.1 h.
RAID AND RAIT OF CEA-EXPRESSING TUMORS

Whole-Body and Red Marrow Doses. The differences found in blood and whole-body half-lives between different kinds of CEA-expressing cancers directly influenced the absorbed doses calculated for the whole body and red marrow. Colorectal cancer patients had significantly lower red marrow and whole-body doses than patients with other cancer types (red marrow: 2.01 ± 1.07 cGy/mCi versus 4.25 ± 1.64 cGy/mCi, P < 0.001; whole body: 0.58 ± 0.30 cGy/mCi versus 1.01 ± 0.36 cGy/mCi, in colorectal versus other cancer patients, respectively; P < 0.001).

Despite the fact that differences in red marrow doses were less pronounced with higher protein doses, deviations of more than 30% of what would have been predicted from the diagnostic study were observed in only 7 (22.6%) of 31 colorectal cancer and in 11 (28.9%) of 38 non-colorectal cancer patients. Although there was a good correlation between the diagnostic and the therapeutic studies (r = 0.7—0.8), there was a tendency of the low-protein, diagnostic injection to overestimate slightly the actual dose measured after the high-protein study (Fig. 7). In not a single case was the absorbed dose observed in the high-protein therapy study more than 25% higher than predicted by the low-protein-dose study.

Organ Dosimetry. Similar differences between colorectal cancer and other tumor types, as were observed in red marrow and whole-body doses, were found in organ doses as well. Colorectal cancer patients had significantly lower liver (e.g., for 131I-labeled MN-14 IgG, 1.81 ± 0.58 cGy/mCi versus 3.74 ± 1.61 cGy/mCi; P < 0.001) and lung doses (e.g., for 131I-labeled MN-14 IgG, 2.04 ± 0.94 cGy/mCi versus 4.40 ± 1.65 cGy/mCi; P < 0.001) than other types of cancer, respectively. The differences in other organ doses showed a similar trend.

Diagnostic Accuracy in Relationship to the Protein Dose. Minimal differences were found in detection sensitivities between the low- and high-protein-dose studies. Only in 3 of the 69 patients were additional lesions detected by the higher protein doses, which were absent in the previous study. In some additional cases, tumor visualization was slightly better (higher tumor:nontumor ratios) with a higher protein dose (compare Figs. 6, 8, and 9). This is due to the higher count rate and thus better imaging statistics of the higher activity administered in the high-protein-dose study and not to the protein dose itself.

Pharmacokinetics of Anti-CEA Antibodies in Comparison with Mu-9, an Antibody That Does Not Recognize a Circulating Epitope. In support of our hypothesis that CEA itself is responsible for the rapid mAb clearance observed in colorectal cancer patients and not a potential influence of the tumor on general protein or antibody metabolism, we compared colorectal cancer patients who underwent
RAID and RAIT with MN-14 and NP-4 to a disease- and stage-matched cohort of patients examined with the anti-CSAp antibody Mu-9. Mu-9 recognizes a conformation-dependent epitope of CSAp that is not measurable in the circulation (36, 38). Fig. 10 shows that, at comparable CEA serum levels, Mu-9 had significantly longer blood (41.7 ± 12.4 h versus 17.6 ± 12.6 h for anti-CSAp versus anti-CEA; \( P < 0.00001 \)) and whole-body half-lives (114.6 ± 50.0 h versus 53.2 ± 30.1 h for anti-CSAp versus anti-CEA; \( P < 0.00001 \)) than the respective anti-CEA mAbs. In contrast to the significant decrease \((P = 0.0003)\) of the blood half-life of the anti-CEA antibodies with increasing plasma CEA levels, no such tendency was seen with Mu-9 (the slope of an equivalent regression line is, at \( P = 0.558 \), not significantly different from 0).

In three patients, Mu-9 (1 mg) was compared intrindividually with MN-14 at low- and high-protein doses (Table 2). Two of these patients suffered from liver and lung metastases of colorectal cancer. In both, blood and whole-body half-lives of Mu-9 were at least twice as high as those of MN-14, whereas the influence of the MN-14 protein dose was minimal. One patient (1585) had only a solitary lung metastasis and a CEA plasma level of only 2.6 ng/ml. He had a nearly identical pharmacokinetic behavior of both mAbs, independent of the protein dose. Fig. 8 shows patient 1575, a 50-year-old woman with liver metastases of colorectal cancer and a plasma CEA level of 246 ng/ml. The highest absolute tumor uptake was seen with Mu-9 (Fig. 8b) because of its longer dwelling time in the blood, allowing the most prolonged targeting. However, the highest tumor:nontumor ratios were achieved with the high-protein dose of MN-14 (Table 2; Fig. 8a), because of a more rapid whole-body clearance than was observed with Mu-9.

**Tumor Dosimetry.** With both anti-CEA mAbs, an inverse logarithmic relationship was observed between the tumor mass and tumor uptake, as well as the radiation dose achieved, as was described for NP-4 earlier (43). No such relationship was found with Mu-9, which recognizes an epitope of a mucin antigen that is more accessible in partially necrotic areas of larger tumors. Overall, no statistically significant difference was seen in the tumor uptake of MN-14, NP-4, or Mu-9.

When comparing the tumor doses and tumor:red marrow ratios between low- and high-protein-dose studies with NP-4 and MN-14, the differences between both studies were mostly less than 25%. However, there were also differences in tumor doses and tumor:red marrow ratios between different types of cancer. The highest tumor:red marrow ratios were achieved in MTC (7.5 ± 3.3; \( n = 11 \)) when compared with most other (non-colorectal) tumor types of comparable sizes (3.2 ± 2.2; \( n = 19 \); \( P < 0.05 \)). Doses that were similarly high as in MTC were found in those colorectal cancer patients with slow, usually monoexponential antibody serum clearance, whereas the tumor:red marrow ratios in rapidly clearing colorectal cancer patients, as well as the other types of CEA-expressing tumors, were mostly less than 5. A more detailed study of factors influencing tumor dosimetry will be published elsewhere.\(^5\)

**DISCUSSION**

In this report, a comparison is made of the pharmacokinetic behavior and biodistribution of one anti-CSAp and two anti-CEA antibodies to determine the factors that influence these parameters. Of particular importance in this comparison are the different affinities of the two anti-CEA mAbs and the difference between a mAb that recognizes a circulating antigen versus one that does not. Patients were selected who had undergone two studies at different protein-dose levels to assess also, on an intraindividual level, how the protein dose may play a role in defining the targeting behavior of the mAbs. Because CEA is expressed in several different cancer types, another issue addressed in this report is whether any difference exists between them with regard to antibody pharmacokinetic behavior. Patients with elevated HAMA titers were excluded for this purpose; the influence of HAMA has been analyzed earlier in several studies (6, 27-30).\(^4\)

Whereas several previous studies, preclinical as well as clinical, have attempted to address the relationship of circulating antigen and protein dose (Refs. 12-26; reviewed in Ref. 10) as well as of a range of other biophysical variables (50-53) in noncirculating (melanoma) and circulating (CEA) systems on an interindividual level, there has been no detailed study analyzing which other factors may affect the pharmacokinetics, imaging sensitivity, and dosimetry of anti-CEA mAbs. Also, the effect of protein dose can be studied best on an intraindividual basis (54).

Patt et al. (18) reported an increasing sensitivity in the detection of liver metastases with increasing protein doses of the \( ^{111}\)In-labeled anti-CEA antibody ZCE-025, up to a dose of 40 mg, with subsequent decreasing sensitivity at higher amounts of protein. Similar observations on increasing serum half-lives, decreasing hepatic uptake, and increasing lesion sensitivities were published by Lamki et al. (22), Murray et al. (19, 20, 21).

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RAID AND RAIT OF CEA-EXPRESSING TUMORS

Fig. 8. Patient 1575, a 50-year-old woman with liver metastases of colorectal cancer (plasma CEA, 246 ng/ml). a, intraindividual comparison of $^{111}$I-labeled MN-14 IgG at a low- and high-protein dose (8 mCi versus 105 mCi) versus Mu-9 at a low protein dose (15 mCi). All scans (200 kilocounts each) were taken at 168 h p.i. and are displayed at the same intensity. Whereas the highest absolute tumor uptake is seen with Mu-9, the best tumor: nontumor ratios are achieved with the high-protein dose of MN-14. b, organ and tumor kinetics of the three injections show at least a biexponential clearance for MN-14 but a monoexponential one for Mu-9. The increased residence time with a higher protein dose of MN-14 is reflected by the higher organ and tumor uptake values at all time points.

Filled symbols, low-protein studies; open symbols, high-protein studies.

20), and Halpern et al. (21) for anti-CEA and anti-melanoma antibodies. However, these studies addressed only the question of sensitivity for diagnostic purposes and compared different protein doses interindividually. Because of the fact that indium was used as the label, no dosimetry was reported, and most importantly, no pathophysiological mechanism was suggested by these authors for their findings. With an abundantly present antigen such as CEA, antigen saturation should not occur at such low mAb protein doses (13, 16).

Pimm (10) has recently reviewed studies on the influence that circulating antigen (CEA and others) may have with respect to targeting sensitivity and whole-body kinetics. He concluded that in humans, there is no appreciable influence, in contrast to animal experiments where, depending upon the model used, contradictory results have been obtained. Also, Bosslet et al. (55) reported no relevant interaction between circulating antigen and the injected anti-CEA antibody in humans. They explained this finding by conformational differences in antigenicity between cell-bound and circulating CEA, as was also reported by Sharkey et al. (24) for the anti-CEA mAb NP-2. In contrast, in animals, some authors found rapid hepatic clearance of immune complexes between the circulating CEA and the
Surprisingly, at similar levels of plasma CEA, colorectal cancer patients cleared the antibody significantly faster from the blood and whole body, with the consequence of lower red marrow, organ, and whole-body doses than patients with most other types of CEA-expressing cancers. This probably reflects differences in the chemical structure of the CEA produced by different kinds of cancer. Our study suggests that there are tumor type-specific heterogeneities in the structure of CEA, which may be reflected by different clearance rates. In fact, several publications on the structure of the oligosaccharide side chains of CEA have reported differences on the basis of individual tumor and patient sources of the investigated molecule. Hernando et al. (60) demonstrated, by immunological methods, differences in the glycosylation pattern between colorectal, stomach, and breast cancer, although the number of specimens examined was too low to permit more general conclusions. Chemical structural analysis of the oligosaccharide side chains also indicated structural heterogeneity,

injected antibody with subsequent metabolic breakdown of these complexes, whereas others did not observe any major influence (13–17, 26). However, animal data cannot sufficiently predict the behavior of CEA complexes in humans, because CEA does not naturally occur in rodents. In humans, a CEA-specific receptor has been described that likely plays a role in its clearance from the blood (56), presumably in addition to more nonspecific mechanisms, such as binding to carbohydrate-specific receptors. The latter is probably the only clearance mechanism available in animals (26, 56, 57).

Despite the difference in affinity by one order of magnitude between MN-14 and NP-4, only very little difference in the pharmacokinetic behavior with regard to clearance, processing, and tumor targeting of both antibodies was found. This is in contrast to published animal data (14, 58, 59). Presumably, the turnover of immune complexes of CEA in colorectal cancer is so rapid that such subtle differences do not cause a dramatic effect. The sometimes striking rapid metabolism of the anti-CEA mAbs despite a relatively low degree of complexation in the 1-h plasma is probably also due to this rapid metabolism of formed complexes, thereby avoiding substantial complex build-up in the blood.

![Fig. 9. Patient 1487, a 65-year-old woman with liver metastases and peritoneal implants of a colon cancer (plasma CEA, 284 ng/ml), is an example of a patient where better count statistics (higher activity given to patients receiving higher protein doses in therapeutic settings), not the higher protein dose itself, led to better detectability of lesions.](image)

![Fig. 10. Blood and whole-body half-lives related to the CEA serum level for both anti-CEA antibodies in comparison with Mu-9.](image)

<table>
<thead>
<tr>
<th>Patient, age, sex</th>
<th>Site of primary tumor</th>
<th>Extent at inj. time</th>
<th>CEA (ng/ml)</th>
<th>mAb</th>
<th>Dose (mg)</th>
<th>Blood t_{1/2} (h)</th>
<th>Whole-body</th>
<th>Tumor</th>
<th>Ratio</th>
<th>T/red marrow</th>
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<tr>
<td>1575 50 yr, F</td>
<td>Sigmoid colon</td>
<td>Liver and lung metastases</td>
<td>246</td>
<td>MN-14</td>
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<td>12.4</td>
<td>2.09</td>
<td>50.4</td>
<td>0.72</td>
<td>4.75-6.55</td>
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<td></td>
<td></td>
<td></td>
<td>MN-14</td>
<td>8.4</td>
<td>12.5</td>
<td>2.71</td>
<td>48.5</td>
<td>0.69</td>
<td>6.68-10.17</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mu-9</td>
<td>1.0</td>
<td>48.7</td>
<td>6.66</td>
<td>119.0</td>
<td>1.54</td>
<td>22.85-34.21</td>
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<tr>
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<td></td>
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<td></td>
<td>Mu-9</td>
<td>0.5</td>
<td>50.4</td>
<td>5.45</td>
<td>171.2</td>
<td>1.06</td>
<td>10.52-43.75</td>
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<td>2.91</td>
<td>104.7</td>
<td>0.67</td>
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<td>3.84</td>
<td>106.2</td>
<td>0.69</td>
<td>6.70</td>
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</tbody>
</table>

* In the case of presenting data for several tumors, the range is given. The same tumors were examined in each study.

* Not determined, since only one imaging time point could be obtained because of the patient's critical condition.
and there is also variability in the carbohydrate moiety attachment sites (56, 61, 62, 65). There has already been speculation, 20 years ago, that tumor type-specific CEA may exist (56, 63). Furthermore, different CEA subtypes have been shown to be cleared at different rates from the blood by the liver (26, 56, 64, 65). Thus, if the immune complexes of CEA and antibody follow the metabolic pathway of CEA, it seems likely that different rates of clearance may be encountered in patients with different CEA-producing tumors. The lack of a pronounced uptake in the spleen and bone marrow, which are two well-known sites of immune complex clearance (e.g., as with HAMA; Refs. 6 and 27–29), supports our view that the clearance is mediated by CEA receptors, not Fc-binding sites. The normal clearance rates of the anti-CSA p antibody Mu-9 in colorectal cancer patients, which is directed against a noncirculating mucin epitope (36, 38), favors further the hypothesis that CEA is the driving force that pulls the antibody out of the serum, not a fundamental metabolic difference between colorectal cancer and other CEA-expressing tumor patients.

The fact that, with fragments, the shorter half-lives in colorectal cancers seemed to be less and the effect of longer $T_{1/2}$ (e.g., in MTC) to be more pronounced than with IgG is easily explained by our hypothesis that the CEA moiety of the complex is the half-life-determining entity. A half-life between 10 and 20 h, as is typical in colorectal cancer patients, is dramatically short for an IgG, although it is normal for a F(ab)$_2$, whereas a half-life between 40 and 50 h, as is typical for MTC, is normal for IgG but three times the typical half-life of F(ab)$_2$.

Why colorectal patients with liver metastases are especially at high risk for rapid clearance remains unclear at this time. Also speculative is the role of the observed biliary and hepatic dysfunction. It is conceivable that they are causally related to the clearance behavior or just epiphenomena of a third component (66), although in animal models, there has been an indication that biliary damage may influence the CEA clearance (56).

Because of the significantly lower whole-body and red marrow doses in colorectal cancer patients with elevated plasma CEA, it is to be expected that the maximum tolerated dose of radiolabeled anti-CEA mAbs in this subset of patients will be higher than in patients with other types of CEA-expressing tumors. Indeed, the results of a Phase II/II clinical RAIT trial with $^{131}$I-labeled FP-4 IgG confirm this assumption (6). On the other hand, because of the unpredictable clearance situation in any individual case, these data clearly favor an individual, dosimetry-based treatment regimen and not just simple mCi/m$^2$ dosing. Encouraging in this context is the fact that, despite a 10-fold difference in protein dose, the diagnostic study could predict the therapeutic dosimetry fairly well. This offers the advantage of a lower risk of HAMA induction by the diagnostic study, which in turn would jeopardize the effectiveness of the therapy (6).

No attempt of an intraindividual comparison of the organ dosimetry with low- and high-protein doses was made, because the therapeutic organ dosimetry is not reliable in the case of a bi- or more-exponential clearance behavior (compare Figs. 4c and 9b), because monoexponential back-extrapolation from the last phase of a biexponential model has already been speculated, 20 years ago, that tumor type-specific CEA may exist (56, 63). Furthermore, different CEA subtypes have been shown to be cleared at different rates from the blood by the liver (26, 56, 64, 65). Thus, if the immune complexes of CEA and antibody follow the metabolic pathway of CEA, it seems likely that different rates of clearance may be encountered in patients with different CEA-producing tumors. The lack of a pronounced uptake in the spleen and bone marrow, which are two well-known sites of immune complex clearance (e.g., as with HAMA; Refs. 6 and 27–29), supports our view that the clearance is mediated by CEA receptors, not Fc-binding sites. The normal clearance rates of the anti-CSA p antibody Mu-9 in colorectal cancer patients, which is directed against a noncirculating mucin epitope (36, 38), favors further the hypothesis that CEA is the driving force that pulls the antibody out of the serum, not a fundamental metabolic difference between colorectal cancer and other CEA-expressing tumor patients.

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