Abstract

Purpose: This phase I study explored the biodistribution and pharmacokinetics of the immunoconjugate CMD-193 (a humanized anti–Lewis Y (LeY) antibody conjugated with calicheamicin) in patients with advanced cancers expressing the LeY antigen.

Experimental Design: The primary objectives were to determine biodistribution and pharmacokinetics of CMD-193. Secondary objectives included response rates and change in tumor metabolism. Patients with progressive, measurable, and LeY positive malignancies were eligible for enrollment in one of two dose cohorts, 1.0 and 2.6 mg/m². The first cycle was trace labeled with ¹¹¹In for biodistribution assessment using γ camera imaging. Subsequent cycles were administered every 3 weeks up to a maximum of six cycles, depending on toxicity and response. Pharmacokinetic analysis was based on radioassay and ELISA.

Results: Nine patients were enrolled in the study. Biodistribution images showed initial blood pool activity, followed by markedly increased hepatic uptake by day 2, and fast blood clearance in all patients. There was low uptake in tumor in all patients. The overall T½β of ¹¹¹In-CMD-193 was 102.88 ± 35.67 hours, with no statistically significant difference between the two dose levels. One patient had a partial metabolic response on ¹⁸F-fluorodeoxyglucose-positron emission tomography (¹⁸F-FDG PET) after four cycles, but no radiological responses were observed. Myelosuppression and effects on liver function were the most significant adverse effects.

Conclusions: CMD-193 shows rapid blood clearance and increased hepatic uptake compared with prior studies of the parental antibody hu3S193. These results highlight the importance of biodistribution and pharmacodynamic assessment in early phase studies of new biologics to assist in clinical development.

The concept of antibody-targeted chemotherapy was successfully translated into the clinic in 2000, when gemtuzumab ozogamicin (Mylotarg, also known as CMA-676) was approved by the Food and Drug Administration for relapsed acute myeloid leukemia (1). This CD33-targeted immunoconjugate of calicheamicin has accelerated the investigation of this therapeutic strategy in solid tumors. CMD-193 is one such calicheamicin immunoconjugate that makes use of the same drug-linker combination as that used in Mylotarg, but the antibody to which it is conjugated targets the Lewis Y (LeY) antigen.

LeY (CD174) is a difucosylated tetrasaccharide internalized antigen displayed on both glycolipid and glycoprotein backbones of membrane surface molecules, and is involved in cellular motility and adhesion (2). LeY has restricted normal tissue expression.
Translational Relevance

This article describes a phase I trial of a novel recombinant immunoconjugate against the Lewis-y antigen, expressed on solid tumors. The results of this study showed the impact of toxin conjugation on the biodistribution and tumor uptake of the immunoconjugate, which could not be predicted based on preclinical studies, and which was not evident from pharmacokinetics or adverse event profiles. Alterations in immunoconjugate biodistribution may impact on tumor uptake and efficacy and, without detailed characterization, will ultimately lead to reduced efficacy and futile clinical trials. This trial design, and results, provides unique information on the importance of pharmacodynamic/bioimaging studies early in the clinical development of immunoconjugates in cancer patients.

expression, and is overexpressed in the majority of epithelial carcinomas (40–90%) including breast, ovary, pancreas, prostate, colon, and lung cancers (3–11).

The immunoconjugate CMD-193 is composed of G193, a humzized monoclonal antibody based on the anti-Ley antibody hu3S193 (12), covalently linked to NAC-γ calicheamicin DMH via an acid-labile AcBut linker with retention of Leγ affinity. In prior phase I trials, hu3S193 showed specific targeting of Leγ-expressing tumors, restricted normal tissue distribution, a long serum half-life, and lack of immunogenicity (13, 14). Hu3S193 is currently under development in phase II trials as a naked humanized antibody in Leγ-expressing tumors. Dose-dependent regression of Leγ-expressing human carcinoma xenografts by the immunoconjugate CMD-193 has been shown in preclinical studies, highlighting the potential therapeutic potential of CMD-193 in cancer patients (15). Initial clinical development of CMD-193 involved a dose escalation phase I study in patients with Leγ-expressing advanced solid tumors with an expanded preliminary evaluation of efficacy in patients with non-small cell lung carcinoma.

In support of the initial clinical development of CMD-193, we conducted an additional phase I dose escalation study of CMD-193 in patients with advanced solid tumors expressing the Leγ antigen. The primary objectives of this trial were to determine the biodistribution and pharmacokinetics of 111In-CMD-193. The tumor uptake of 111In-CMD-193 was based on qualitative and quantitative assessment of biodistribution images and dosimetry. Secondary objectives were to determine tumor response to CMD-193 through changes in tumor 18F-FDG PET metabolism and measurement by Response Evaluation Criteria in Solid Tumors (RECIST) criteria (16).

Materials and Methods

Patients. Eligible patients were ≥18 y of age, who had histologically confirmed solid malignancies with ≥20% tumor cells displaying Leγ antigen positivity on immunohistochemistry of archived tumor samples (13), and who had progressed following standard therapy. Inclusion criteria included the following: measurable disease, Eastern Cooperative Oncology Group performance status of 0 to 1, and adequate renal, hepatic, and bone marrow function and ability to give informed consent. Exclusion criteria included the following: cancer therapy within 21 d of the first dose of CMD-193, clinically active central nervous system metastases, significant prior allergic reaction to recombinant human or murine proteins, and serious concurrent medical conditions including chronic liver disease.

Trial design. This trial planned to enroll into three dose cohorts: 1.0, 1.7, and 2.6 mg/m². These dose levels were selected based on the lowest practical dose able to be trace labeled for biodistribution studies (1.0 mg/m²), and the anticipated therapeutic dose based on other calicheamicin conjugates. After a parallel phase I trial determined the maximum tolerated dose of CMD-193 to be 3.6 mg/m² and therapeutic dose to be 2.6 mg/m², with dose limiting toxicity related to thrombocytopenia and hepatic enzyme changes, a protocol amendment was approved to remove the 1.7 mg/m² cohort. Following pretreatment assessments, eligible patients received a single infusion of Indium-111-labeled CMD-193 [111In-CMD-193; 3-7 mCi (120-280 MBq)] at a protein dose level of 1.0 or 2.6 mg/m² over 1 h on day 1 of cycle 1. Subsequent cycles of unlabeled CMD-193 were administered at three weekly intervals up to a maximum of six cycles, subject to tolerability and response.

Radiolabeling of CMD-193. The immunoconjugate CMD-193 (Wyeth Pharmaceuticals, Inc.) was labeled with 111In-(MDS Nordion) via the bifunctional metal ion chelate CHX-A*-diethylenetriaminopentaaceic acid according to methods described previously (12, 17).

Assessments. Biodistribution evaluation was done by whole body γ camera scans on day 1, day 2, day 3 or 4, day 5 or 6, and day 7 or 8 following 111In-CMD-193 infusion. Pharmacokinetic sampling of 111In-CMD-193 was done during cycle 1 on day 1 (preinfusion, 1 and 4 h postinfusion commencement), day 3, day 8, and day 15. Changes in tumor metabolism were evaluated using 18F-FDG-PET, which was done at screening, between days 15 and 21 of cycles 2 and 4, and at study completion. Antitumor response was assessed using RECIST criteria (16), with computed tomography (CT) scans performed at screening, between days 15 and 21 of cycles 2 and 4, and at study completion. Safety evaluation was done weekly throughout the trial with all adverse events documented and graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Causality was determined as “related to study drug” if the event was deemed definitely, probably, or possibly related to the administration of CMD-193 by the investigator.

Biodistribution and whole body clearance. Whole body planar γ camera images were acquired on a dual-headed γ camera (Picker International and Phillips Medical Systems). Single photon emission CT (SPECT) images of a region of the body with known tumor were also obtained on at least one occasion during this period. Biodistribution analysis was done by examination of whole body and SPECT images by experienced nuclear medicine physicians. Comparison to prior studies of parental antibody hu3S193 was also done (13). Whole body and organ clearance of 111In-CMD-193 was calculated from quantitative whole body conjugate view γ camera images obtained at the multiple time points postinfusion using a well-validated method (13, 18, 19).

Pharmacokinetics. Serum obtained from patients following infusion of 111In-CMD-193 was aliquoted and counted in a γ scintillation counter (Packard Instruments). The results were expressed as % injected dose per liter (%ID/L) and µg/mL. Estimates were determined for the following parameters: T/α and T/β (half-lives of the initial and terminal phases of disposition); V1, volume of central compartment; Cmax maximum serum concentration; AKC, area under the serum concentration curve extrapolated to infinite time; and CL, total serum clearance. A two-compartment i.v. bolus model with macroparameters, no lag time, and first-order elimination (WNL Model 8) was fitted to individual serum. 111In-CMD-193 data for each subject using unweighted nonlinear, least squares with WinNonLin version 5.2 (Pharsight Corp).

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Measured serum levels of CMD-193 were expressed as ng/mL. Serum samples were also obtained for measurement of total and free calcilcemin by a validated ELISA protocols (LLOQ, 2.45 ng/mL).

**Tumor metabolic response assessment.** Patient preparation for and acquisition of FDG-PET scans were standardized (20, 21). For each FDG-PET performed, the maximum standardized uptake value (SUVmax) corrected for body weight for all target lesions >2 cm identified on CT imaging was calculated using region of interest. The region of interest was determined with the aid of the anatomic detail provided on CT imaging was calculated using region of interest. The region of interest for normal lung tissue was taken as the reference region for each patient, which ensured that any SUV changes in the independent samples test were significant different between dose levels (P = 0.74). Quantitative analysis confirmed the high levels of hepatic uptake of 111In-CMD-193 apparent visually on biodistribution images. Compared with the parental hu3S193 antibody, hepatic uptake was significantly higher at 24 hours (time of maximal hepatic uptake; Table 2). Tumor uptake was also significantly lower for CMD-193 compared with hu3S193 (Table 2).

**Pharmacokinetics and HAHA.** CMD-193 displayed a fast clearance from blood, consistent with the biodistribution findings (Table 3). Selected serum samples from patients were analyzed by fast protein liquid chromatography for immune complex or metabolite formation, and no complexes or metabolites were found for T1/2α, T1/2β, clearance, or V1 between the two dose levels. The results for CMD-193 were significantly different compared with the parental antibody hu3S193 (13), with T1/2β for CMD-193 102.88 ± 35.76 hours versus 22.09 ± 9.87 hours for hu3S193 (P < 0.001) and CL for CMD-193 113.22 ± 56.58 mL/h versus 22.09 ± 9.87 mL/h for hu3S193, P < 0.001. No HAHA was detected in any patient. Free calcilcemin levels were at or below the limit of assay quantitation in all patients.

**Results.**

**Patient characteristics.** Nine patients were eligible and enrolled (six patients, 1.0 mg/m² cohort; three patients, 2.6 mg/m² cohort). Baseline patient demographics and disease characteristics are shown in Table 1. All patients had metastatic disease at study entry and many patients had been extensively pretreated, having received one to five lines of prior chemotherapy, monoclonal antibody, or biological agent. Only one patient (patient 102) received all six cycles of CMD-193. Four patients were withdrawn due to progressive disease after two cycles of treatment (patients 101, 105, 106, and 161), and four patients were withdrawn because of toxicity (patients 103, 104, 107, and 108).

**Biodistribution.** Evaluation of γ camera imaging following infusion of 111In-CMD-93 showed rapid clearing of blood pool activity, followed by markedly increased hepatic uptake by day 2, persisting to day 8 (Fig. 1). This pattern was observed for all patients in both dose levels. No significant uptake of 111In-CMD-193 in tumor was visualized in target lesions for all patients.

Whole body clearance was (mean ± SD) 47.82 ± 3.24 hours, and there was no statistically significant difference between dose levels (P = 0.74). Quantitative analysis confirmed the high levels of hepatic uptake of 111In-CMD-193 apparent visually on biodistribution images. Compared with the parental hu3S193 antibody, hepatic uptake was significantly higher at 24 hours (time of maximal hepatic uptake; Table 2). Tumor uptake was also significantly lower for CMD-193 compared with hu3S193 (Table 2).

**Table 1. Patient characteristics at study entry and study outcomes**

<table>
<thead>
<tr>
<th>Pt ID</th>
<th>Age (y)</th>
<th>Sex</th>
<th>ECOG</th>
<th>Primary diagnosis</th>
<th>Sites of metastatic disease at study entry</th>
<th>Tumor Leγ antigen positivity</th>
<th>Dose cohort</th>
<th>No. of cycles</th>
<th>FDG-PET response (EOS)</th>
<th>CT response (EOS)</th>
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<td>PD</td>
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<td>6</td>
<td>SMD</td>
<td>PD</td>
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<tr>
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<td>4</td>
<td>PMR</td>
<td>SD</td>
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<td>3</td>
<td>SMD</td>
<td>SD</td>
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<td>1</td>
<td>2</td>
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<td>2</td>
<td>2*</td>
<td>N/A</td>
<td>PD*</td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; EOS, end of study assessment; N/A, not assessed.

*Withdrew prematurely cycle 2 day 9. Response assessed clinically, as patient died of progressive disease before repeat CT scan.
end of study assessment. Patient 108 showed SMD, but had a 25% reduction in SUV_{max} (prestudy, 4.4; restaging, 3.3) after five cycles of treatment. Of the four patients with PMD, three were in dose cohort 1 and one was in dose cohort 2. Assessment of antitumor response by CT scanning showed four patients with stable disease (SD) and four with progressive disease (PD) at final staging.

**Adverse events.** CMD-193 at doses of 1.0 and 2.6 mg/m² was reasonably well tolerated, and no difference in toxicity was observed between patients in the two dose levels (Table 4). Four patients, two from each dose cohort, were withdrawn because of toxicity. The main adverse events with some relationship to CMD-193 were asymptomatic myelosuppression, particularly thrombocytopenia (12 grade 1-2 events, 2 grade 3 events, and 1 grade 4 event), and abnormal liver function (5 grade 3 events). All other related adverse events, including fatigue, lethargy, anorexia, and nausea, were mild (grade 1-2). There were no infusion-related reactions. There were no serious adverse events related to study drug, and no serious or severe

| Table 2. Peak tumor uptake and liver uptake of CMD-193 compared with parental antibody hu3S193 |
|----------------------------------|----------------------------------|
|                                  | hu3S193                          | CMD193                          |
| Peak tumor uptake (μg/gm)       | Mean ± SD 2.9 ± 1.7               | Mean ± SD 0.15 ± 0.03*           |
| Liver %ID 24 p.i.               | 7.3 ± 1.5                        | 33 ± 6.0†                       |

*Peak tumor uptake hu3S193 vs CMD-193, \( P = 0.0016 \).
†Maximal liver uptake hu3S193 vs CMD-193, \( P < 0.0001 \).
unexpected toxicities were observed. Toxicity was consistent with that found in the parallel phase I trial of CMD-193.9

**Discussion**

This study showed lack of targeting of CMD-193 to known sites of metastatic disease, and marked hepatic uptake and rapid clearance from blood, consistent with the observed short T$_{1/2}$β and fast serum clearance. There were no documented objective responses seen in size of tumor, but one patient did display a PMR according to 18F-FDG-PET analysis. These bioimaging and pharmacokinetic results highlight the importance of detailed investigation of the properties of antibodies and immunoconjugates in early phase I trials, and can provide critical information impacting on subsequent clinical development.

The biodistribution, clearance, and pharmacokinetic properties of CMD-193 were found to be significantly different to the parental antibody hu3S193. Phase I studies of 111In-hu3S193 have shown prominent specific uptake in tumor, a lack of consistent normal tissue/organ uptake, and a long half-life in blood (13, 14). This is in contrast to the fast clearance from blood, rapid uptake in liver parenchyma, and lack of tumor uptake of 111In-CMD-193 observed in this study. Importantly, one patient (patient 103) participated in both clinical studies, allowing direct comparison of biodistribution, clearance, and hepatic uptake between CMD-193 and hu3S193 in the same patient (Fig. 3). The whole body clearance and terminal half-life of 111In-CMD-193 were also faster than that observed with other

| Table 3. 111In-CMD-193 serum pharmacokinetic analysis results |
|-------------|----------------|----------------|----------------|----------------|
| Parameter   | Units          | CMD-193 All   | 1 mg/m² CMD-193 | 2.6 mg/m² CMD-193 |
|             | (n = 9)        | CMD-193 (n = 6) |                |                |
| T½α         | Hour           | 4.76 ± 2.15   | 5.47 ± 1.99    | 3.33 ± 2.00    |
| T½β         | Hour           | 102.88 ± 35.67| 104.42 ± 37.94 | 99.79 ± 38.32  |
| V1          | mL             | 4,071.22 ± 731.41| 4,366.18 ± 586.87| 3,481.31 ± 704.13 |
| CL          | mL/h           | 113.22 ± 56.58| 139.04 ± 61.25 | 79.56 ± 29.67  |
| AUC         | µg.h/mL        | 29.43 ± 22.31 | 29.43 ± 6.31   | 65.45 ± 17.05  |

Fig. 2. PMR to CMD-193: assessment by 18F-FDG-PET. According to RECIST criteria, patient 103 had stable disease following four cycles of CMD-193 at a dose of 1.0 mg/m², with a large liver lesion (arrow) remaining similar in size on CT. The patient did however show a PMR in this lesion, with a 41.7% reduction in SUV$_{max}$ observed on 18F-FDG-PET. Presudy and post-CMD-193 cycle 4 imaging is shown: A and D, CT image; B and E, 18F-FDG-PET; C and F, fused PET/CT images.
To our knowledge, CMB-401 (hCTM01-calicheamicin) is the only calicheamicin immunoconjugate to have reached phase II trials in solid tumors. This combined N-acetyl/analogue of calicheamicin with a polymorphic epithelial mucin targeting humanized antibody hCTM01 using an amide-based linkage. An initial phase I study in patients with epithelial ovarian cancer, which included a predose of unconjugated antibody to minimize uptake in normal tissues and complex formation with circulating antigen, found it to be tolerable and defined the maximum tolerated dose (27). A subsequent phase II trial in 21 patients with recurrent ovarian cancer failed to show clinical efficacy (28). In this case, a lack of efficacy (and suspension of development) was attributed to instability of the amide linker, although free calicheamicin and measurement

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<td>8</td>
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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Fig. 3. 111In-CMD-193 biodistribution pattern compared with the biodistribution of the parental anti-LeX antibody 111In-hu3S193 observed in the prior phase I study13. Patient 103 had participated in both clinical studies, allowing direct comparison of biodistribution, clearance, and hepatic uptake between CMD-193 and hu3S193 in the same patient. A, 1 d; B, 7 d postinfusion of 111In-CMD-193 (arrow, tumor in liver); C, day 0; D, 2 d; and E, 7 d postinfusion of 111In-hu3S193. Note that in the original hu3S193 trial, a small tumor in the liver was visualized on SPECT imaging.
of serum complex formation results were not published. Bio-
distribution and pharmacokinetic assessment of CMD-401 in
patients were also not done in this trial, and hence, it is not
possible to draw direct comparisons with CMD-193 other
than the common toxicity profile relating to calicheamicin
(29, 30).

CMD-193 showed a similar toxicity profile to Mylotarg and
CMB-401, with predominant hematopoietic and hematological
toxicity. Hepatic toxicity seen in some patients following admin-
istration of CMD-193 may be explained by hepatic uptake and
metabolism of the Nac3- calicheamicin DMH, as Le3 is not ex-
pressed by liver cells (4). With Mylotarg however, hepatotoxic-
ity can be explained partly by sinusoidal obstruction syndrome,
the mechanism of which probably involves targeting of CD33+
cells in the sinusoids of the liver (30). Although liver toxicity
was also documented in phase I study of CMB-401 (but inter-
estingly not mentioned in phase II trial), in this case, it was at-
tributed to expression of target antigen in liver bile duct cells,
rather than to uptake of calicheamicin metabolites by hepato-
cytes (27, 28). Myelosuppression following CMD-193 may be
explained by the myelosuppressive effects of a small amount of
free calicheamicin.

In summary, the detailed biodistribution and pharmacoki-
netic assessment performed in this trial was able to identify an
unexpected in vivo fate for the novel Le3-targeting immunoconj-
guate CMD-193 in patients with advanced Le3-positive epithelial
cancers. Although CMD-193 was generally tolerable, and hints of
biological activity were shown, the marked hepatic uptake, low
tumor uptake, and rapid blood clearance observed was in con-
trast to the parental antibody hu3S193. On the basis of clinical
trial data and the biodistribution results shown in this study, the
clinical development of CMD-193 has not been continued.
These results highlight the importance of detailed biodistribu-
tion and pharmacodynamic assessment in early phase studies
of new biologics to inform and guide clinical development.

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