

Phase I Biodistribution and Pharmacokinetic Study of Lewis Y-Targeting Immunoconjugate CMD-193 in Patients with Advanced Epithelial Cancers

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Abstract Purpose: This phase I study explored the biodistribution and pharmacokinetics of the immunoconjugate CMD-193 [a humanized anti-Lewis Y (Le^y) antibody conjugated with calicheamicin in patients with advanced cancers expressing the Le^y 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 Le^y 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 T_{1/2} β of ¹¹¹In-CMD-193 was 102.88 \pm 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. (Clin Cancer Res 2009;15(21):6709–15)

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 (Le^y) antigen.

Le^y (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). Le^y has restricted normal tissue

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Translational Relevance

This article describes a phase I trial of a novel recombinant immunoconjugate against the Lewis γ 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 humanized monoclonal antibody based on the anti-Le γ antibody hu3S193 (12), covalently linked to NAc- γ calicheamicin DMH via an acid-labile AcBut linker with retention of Le γ affinity. In prior phase I trials, hu3S193 showed specific targeting of Le γ -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 Le γ -expressing tumors. Dose-dependent regression of Le γ -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 Le γ -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 Le γ antigen. The primary objectives of this trial were to determine the biodistribution and pharmacokinetics of ^{111}In -CMD-193. The tumor uptake of ^{111}In -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 ^{18}F -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 $\geq 20\%$ tumor cells displaying Le γ 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, he-

matic, 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/m 2 . These dose levels were selected based on the lowest practical dose able to be trace labeled for biodistribution studies (1.0 mg/m 2), 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/m 2 and therapeutic dose to be 2.6 mg/m 2 ,⁹ with dose limiting toxicity related to thrombocytopenia and hepatic enzyme changes, a protocol amendment was approved to remove the 1.7 mg/m 2 cohort. Following pretreatment assessments, eligible patients received a single infusion of Indium-111-labeled CMD-193 [^{111}In -CMD-193; 3-7 mCi (120-280 MBq)] at a protein dose level of 1.0 or 2.6 mg/m 2 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 ^{111}In - (MDS Nordion) via the bifunctional metal ion chelate CHX-A''-diethylenetriaminepentaacetic 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 ^{111}In -CMD-193 infusion. Pharmacokinetic sampling of ^{111}In -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 ^{18}F -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 ^{111}In -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 ^{111}In -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 $\mu\text{g}/\text{mL}$. Estimates were determined for the following parameters: $T_{1/2\alpha}$ and $T_{1/2\beta}$ (half-lives of the initial and terminal phases of disposition); V_1 , volume of central compartment; C_{max} , maximum serum concentration; AUC, 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 ^{111}In -CMD-193 data for each subject using unweighted nonlinear, least squares with WinNonLin version 5.2 (Pharsight Corp).

⁹ C. Zacharchuk and D.S. Sonnichsen, unpublished data.

Table 1. Patient characteristics at study entry and study outcomes

Pt ID	Age (y)/ Sex	ECOG PS	Primary diagnosis	Sites of metastatic disease at study entry	Tumor Le ^y antigen positivity	Dose cohort	No. of cycles	FDG-PET response (EOS)	CT response (EOS)
101	53/F	1	Colon carcinoma	Lymph nodes, liver	20-50%	1	2	PMD	PD
102	49/F	0	Cholangiocarcinoma	Retro-pancreatic mass	>75%	1	6	SMD	PD
103	71/M	0	Colon carcinoma	Liver	51-75%	1	4	PMR	SD
104	58/M	0	Gastric carcinoma	Liver	20-50%	1	3	SMD	SD
105	53/M	0	Colon carcinoma	Lymph nodes liver, lung, presacrum	20-50%	1	2	PMD	PD
106	46/M	0	Gastro-esophageal junction carcinoma	Lymph nodes, liver	51-75%	1	2	PMD	PD
107	77/M	1	Bronchoalveolar carcinoma	Lung	20-50%	2	1	PMD	SD
108	69/F	0	Gastric carcinoma	Lymph nodes, liver, lung	20-50%	2	5	SMD	SD
161	46/F	1	Pulmonary adenocarcinoma	Lymph nodes liver, bone	20-50%	2	2*	N/A	PD*

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.

Measured serum levels of CMD-193 were expressed as ng/mL. Serum samples were also obtained for measurement of total and free calicheamicin 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 (SUV_{max}) corrected for body weight for all target lesions >2cm identified on CT imaging was calculated using region of interest. The region of interest was determined with the aid of the anatomic detail provided by the CT scan. SUV_{max} for normal lung tissue was taken as the reference region for each patient, which ensured that any SUV changes in tumors could be directly attributed to treatment response or to disease progression (16). Metabolic response was calculated using the target lesion with the greatest baseline SUV_{max} and was categorized according to the European Organization for Research and Treatment of Cancer guidelines (20).

Human-anti-human antibody response. Assay of serum for anti-CMD-193 antibodies in human serum was done by Wyeth Pharmaceuticals using a validated ELISA protocol.

Statistical considerations. The independent samples *t* test was used to compare mean pharmacokinetic and clearance parameters across the two dose cohorts of CMD-193. It was also used to compare results with those obtained with our prior phase I trial using the unconjugated parental antibody hu3S193 (13).

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 \pm SD) 47.82 \pm 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).

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 or free ¹¹¹In-chelate was observed up to 72 hours post-infusion (data not shown). No significant differences were found for T_{1/2} α , T_{1/2} β , clearance, or VI between the two dose levels. The results for CMD-193 were significantly different compared with the parental antibody hu3S193 (13), with T_{1/2} β for CMD-193 102.88 \pm 35.67 hours versus 189.63 \pm 62.17 hours for hu3S193 (*P* < 0.001) and CL for CMD-193 113.22 \pm 56.58 mL/h versus 22.09 \pm 9.87 mL/h for hu3S193, *P* < 0.001. No HAHA was detected in any patient. Free calicheamicin levels were at or below the limit of assay quantitation in all patients.

Response assessment. Metabolic response by FDG-PET and antitumor response according to RECIST criteria results are shown in Table 1. Response could not be formally measured in patient 161, who died of rapid progressive disease following early study withdrawal during cycle 2. Patient 103 showed a partial metabolic response (PMR), with a 41.7% reduction in SUV_{max} (prestudy, 10.8; restaging, 6.3) after four cycles of CMD-193 (Fig. 2). This was despite no change in CT dimensions of the target lesion. Stable metabolic disease (SMD) was observed in three patients and four had progressive metabolic disease (PMD) at

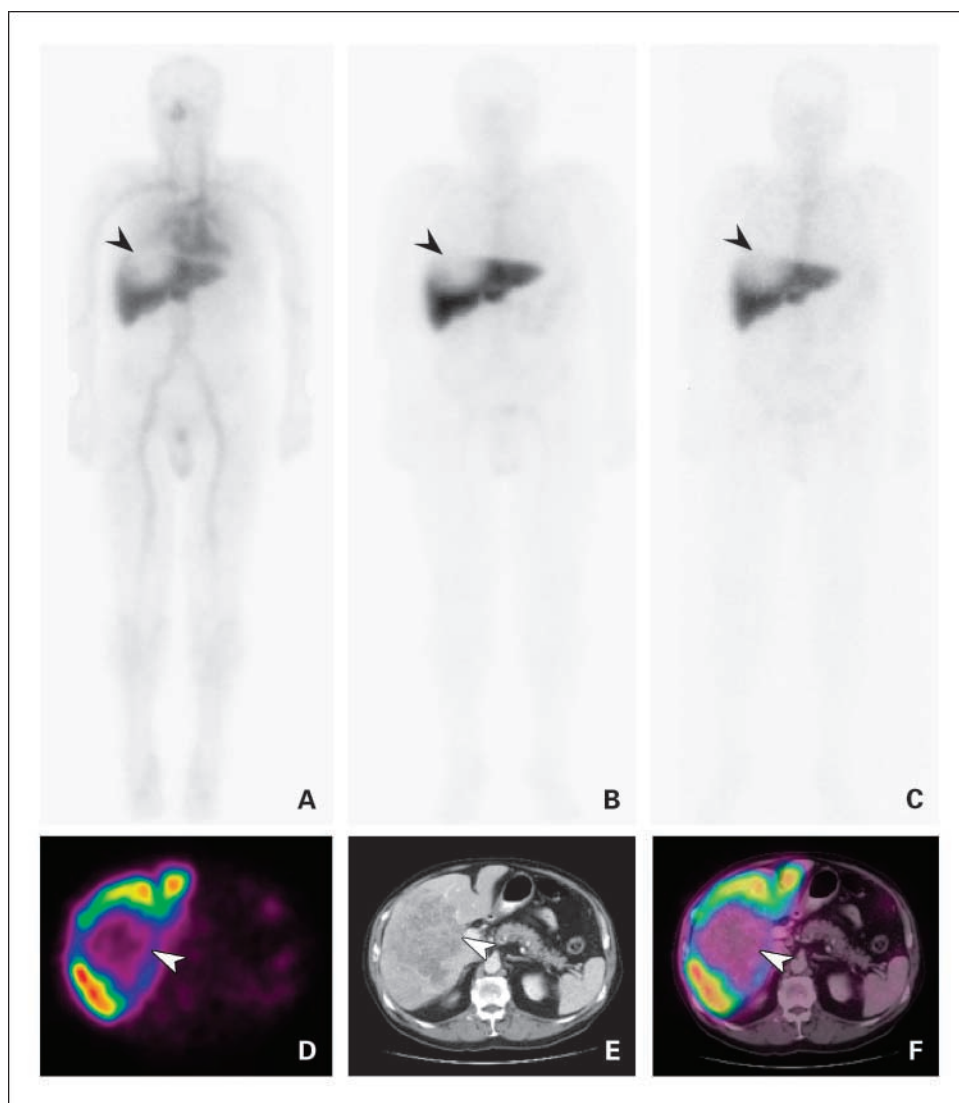


Fig. 1. Representative biodistribution pattern of ¹¹¹In-CMD-193. Anterior whole body γ camera images in patient 106 (1.0 mg/m² dose cohort) following infusion are shown for day 1 (A), day 3 (B), and day 8 (C). Following infusion of ¹¹¹In-CMD-193, there was initial blood pooling, followed by markedly increased hepatic uptake by day 2 that persisted to day 8. No tumor uptake was apparent in the whole body γ camera images (arrow) or SPECT (D). E, corresponding CT scan shows the large hepatic metastasis, also evident in F, coregistered SPECT/CT scan.

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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	
	Mean \pm SD	Range	Mean \pm SD	Range
Peak tumor uptake (μ g/gm)	2.9 \pm 1.7	1.2-6.3	0.15 \pm 0.03*	0.11-0.19
Liver %ID 24 p.i.	7.3 \pm 1.5	4.5-9.5	33 \pm 6.0 [†]	26.9-42.0

*Peak tumor uptake hu3S193 vs CMD-193, *P* = 0.0016.

[†]Maximal liver uptake hu3S193 vs CMD-193, *P* < 0.0001.

Table 3. ¹¹¹In-CMD-193 serum pharmacokinetic analysis results

Parameter	Units	CMD-193 All (n = 9)		1 mg/m ² CMD-193 (n = 6)		2.6 mg/m ² CMD-193 (n = 3)		t test (comparing two dose levels)
		Mean	SD	Mean	SD	Mean	SD	P
T _{1/2} α	Hour	4.76	2.15	5.47	1.99	3.32	2.00	0.17
T _{1/2} β	Hour	102.88	35.67	104.42	37.94	99.79	38.32	0.87
V ₁	mL	4,071.22	731.41	4,366.18	586.87	3,481.31	704.13	0.08
CL	mL/h	113.22	56.58	130.04	61.25	79.56	29.67	0.23
AUC	μg.h/mL	29.93	22.31	16.37	6.13	56.45	17.05	Not done

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

humanized IgG1 antibodies (19, 22). Interestingly, this difference in serum clearance of a toxin-conjugate, compared with the parental antibody (hu3S193), has also been recently reported for the Herceptin-maytansinoid conjugate T-DM1, which had a T_{1/2} of 2.1 to 3.7 days (compared with Herceptin T_{1/2} of >10 days), although biodistribution data were not reported for this conjugate (23, 24).

The rapid clearance of CMD-193 from blood, and the liver uptake observed, was not predicted by preclinical studies. The marked difference in biodistribution of CMD-193 compared with parental antibody hu3S193 cannot be explained by antibody specificity, as retention of Le^y binding by CMD-193 was confirmed before infusion. The lack of CMD-193 complexes or metabolites in blood (measured by fast protein liquid chromatography), absence of HAHA, and the lack of prominent spleen or bone marrow uptake on imaging, excludes CMD-193 complexes or free ¹¹¹In-contributing to the increased liver uptake. It is possible that a physicochemical change induced by conjugation of the antibody with calicheamicin may have led to the altered biodistribution observed in this study. Size and charge are known to influence the uptake of circulating macromolecules by hepatic cells, possibly by influencing electrostatic attraction and hydrophobic interactions or specific receptor-mediated interactions with scavenger receptors, which remove acidic macromolecules (24–26). Despite the low uptake of CMD-193 in tumor, the observation of a PMR in one patient highlights the potent biological effect of calicheamicin, even at low tumor concentrations.

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. Prestudy 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.

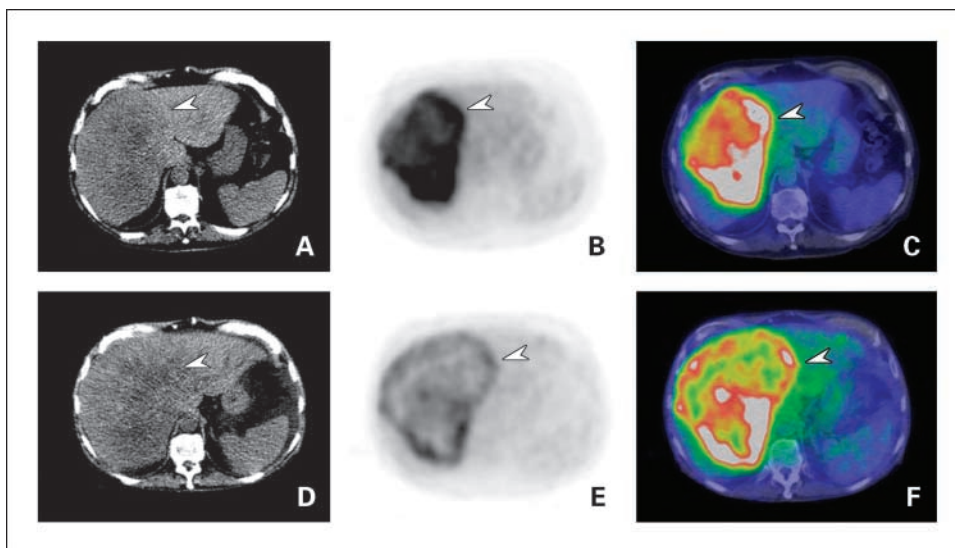


Table 4. CMD-193 related adverse events (possibly, probably, or definitely related)

System organ class	Adverse event	G1	G2	G3	G4	Total
Blood and lymphatic system disorders	Anemia	4	2	0	0	6
	Leucopenia	3	1	0	0	4
	Lymphopenia	3	2	1	0	6
	Neutropenia	1	2	0	0	3
	Thrombocytopenia	11	1	2	1	15
Gastrointestinal disorders	Abdominal bloating	1	0	0	0	1
	Epigastric discomfort	1	0	0	0	1
	Gastresophageal reflux	1	2	0	0	3
	Nausea	8	2	0	0	10
	Vomiting	6	1	0	0	7
General disorders and administration site conditions	Flu-like symptoms	1	0	0	0	1
	Flushing	1	0	0	0	1
	Fatigue	0	2	0	0	2
	Lethargy	6	2	0	0	8
Hepatobiliary disorders	Hyperbilirubinaemia	4	2	0	0	6
Investigations	ALP increased	1	1	2	0	4
	ALT increased	2	3	0	0	5
	Amylase increased	1	0	0	0	1
	AST increased	2	0	1	0	3
	GGT increased	0	1	2	0	3
	Lipase increased	1	1	0	0	2
	Weight loss	0	2	0	0	2
	Anorexia	7	1	0	0	8
Respiratory, thoracic and mediastinal disorders	Epistaxis	1	0	0	0	1
Skin and subcutaneous tissue disorders	Bruising	1	0	0	0	1
	Rash erythematous	1	0	0	0	1
Total		68	28	8	1	105

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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

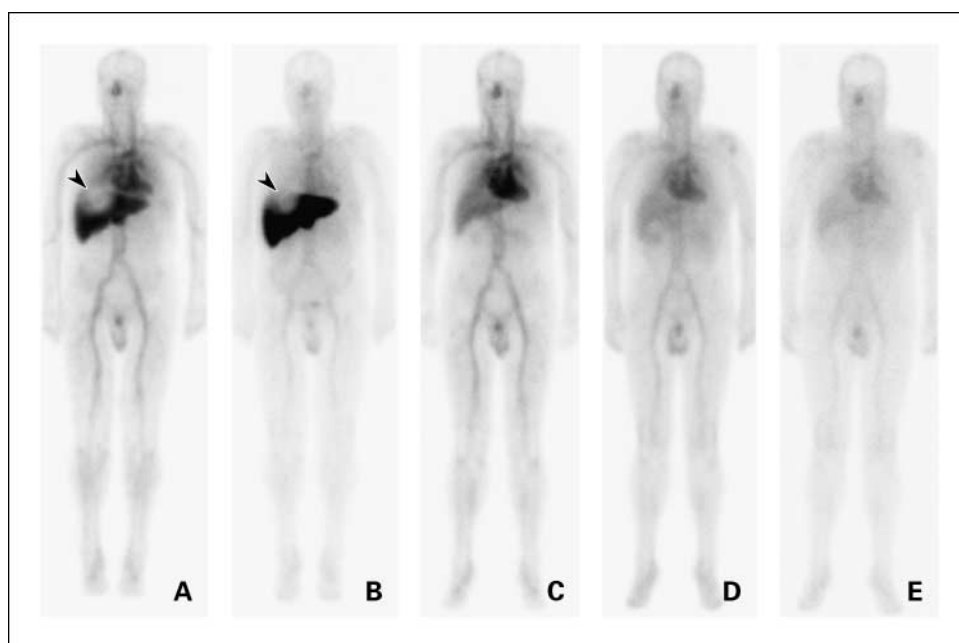


Fig. 3. ¹¹¹In-CMD-193 biodistribution pattern compared with the biodistribution of the parental anti-Le^Y antibody ¹¹¹In-hu3S193 observed in the prior phase I study¹³. Patient 103 had participated in both clinical studies, allowing direct comparison of biodistribution, clearance, and hepatic uptake between 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.

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of serum complex formation results were not published. Biodistribution and pharmacokinetic assessment of CMB-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 hepatic and hematological toxicity. Hepatic toxicity seen in some patients following administration of CMD-193 may be explained by hepatic uptake and metabolism of the NAc- γ calicheamicin DMH, as Le^y is not expressed by liver cells (4). With Mylotarg however, hepatotoxicity 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 interestingly not mentioned in phase II trial), in this case, it was attributed to expression of target antigen in liver bile duct cells, rather than to uptake of calicheamicin metabolites by hepatocytes (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 pharmacokinetic assessment performed in this trial was able to identify an unexpected *in vivo* fate for the novel Le^y-targeting immunoconjugate CMD-193 in patients with advanced Le^y-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 contrast 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 biodistribution and pharmacodynamic assessment in early phase studies of new biologics to inform and guide clinical development.

Disclosure of Potential Conflicts of Interest

D.S. Sonnichsen, employment, Wyeth Research. Funding for this study was provided by Wyeth Research.

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