

A Phase I Biodistribution and Pharmacokinetic Trial of Humanized Monoclonal Antibody Hu3s193 in Patients with Advanced Epithelial Cancers that Express the Lewis-Y Antigen

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Abstract Purpose: We report a first-in-man trial of a humanized antibody (hu3S193) against the Le^y antigen.

Experimental Design: Patients with advanced Le^y-positive cancers received four infusions of hu3S193 at weekly intervals, with four dose levels (5, 10, 20, and 40 mg/m²). The first infusion of hu3S193 was trace labeled with Indium-111, and biodistribution, pharmacokinetics, tumor uptake, and immune response were evaluated in all patients.

Results: A total of 15 patients (7 male/8 female; age range, 42-76 years; 6 breast, 8 colorectal cancer, and 1 non – small-cell lung cancer) were entered into the study. Transient grade 1 to 2 nausea and vomiting was observed following infusion of hu3S193 at the 40mg/m² dose level only. There was one episode of dose-limiting toxicity with self-limiting Common Toxicity Criteria grade 3 elevated alkaline phosphatase observed in one patient with extensive liver metastases. The bio-distribution of ¹¹¹In-hu3S193 showed no evidence of any consistent normal tissue uptake, and ¹¹¹In-hu3S193 uptake was observed in cutaneous, lymph node, and hepatic metastases. Hu3S193 displayed a long serum half-life (T_{1/2β} = 189.63 ± 62.17 h). Clinical responses consisted of 4 patients with stable disease and 11 patients with progressive disease, although one patient experienced a 89% decrease in a lymph node mass, and one patient experienced inflammatory symptoms in cutaneous metastases, suggestive of a biological effect of hu3S193. No immune responses (human anti-human antibody) to hu3S193 were observed.

Conclusion: Hu3S193 is well tolerated and selectively targets tumors, and the long half-life and biological function *in vivo* of this antibody makes it an attractive potential therapy for patients with Le^y-expressing cancers.

The Lewis-y (Le^y) antigen is a blood group-related antigen expressed in over 70% of epithelial cancers (including breast, colon, ovary, and lung cancers) and is an attractive target for monoclonal antibody-directed therapy (1–9). A number of

phase I clinical trials with mouse or humanized anti-Le^y antibodies have been conducted to date. Trials of murine BR55-2 (10, 11), ABL-264 (12), B3 (13), and LMB-1 (murine antibody B3 linked to *Pseudomonas* exotoxin; ref. 14) have been conducted with some minor responses observed; however, immunogenicity of constructs has restricted the use of these antibodies. Recently, a phase I trial of a humanized anti-Le^y antibody IGN311 (based on the murine BR55-2 antibody) was reported to show favorable safety and pharmacokinetic data (15).

A chimeric BR96-doxorubicin construct (16, 17) has also been evaluated in a range of patients with advanced cancers at doses up to 700 mg/m². In clinical trials, upper gastrointestinal toxicity was seen in doses > 200 mg/m², and weak immune responses to BR96-doxorubicin was observed in 37% of patients (18). Phase II trials of BR96-doxorubicin in breast cancer and gastric cancer patients have been performed, with limited clinical activity seen (19, 20). BR96-doxorubicin (SGN-15) has also been evaluated in phase II trials in conjunction with Taxotere in non – small-cell lung carcinoma patients, and an improvement in overall survival compared with Taxotere alone was reported (21). Interestingly, dosing with SGN-15

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prior Taxotere has been shown to have improved the effect assessed by ^{18}F -FDG positron emission tomography scans.

We have developed a CDR-grafted humanized version of murine anti-Le y monoclonal antibody 3S193 (hu3S193), which has undergone extensive preclinical characterization (22–27). Hu3S193 has potent immune effector function [complement-dependent cytotoxicity (CDC), IC_{50} 1.0 $\mu\text{g}/\text{mL}$ and antibody-dependent cellular-cytotoxicity (ADCC), IC_{50} 0.5 $\mu\text{g}/\text{mL}$], is rapidly internalized into Le y expressing cancer cells, and has been shown in preclinical studies alone or in conjunction with isotopes and toxins to cause significant regressions in xenograft models (23–26, 28). We report the results of a first-in-human trial of hu3S193 in patients with Le y -positive epithelial cancers.

Materials and Methods

Trial design

This first-in-human trial was an open-label, dose escalation phase I study. The primary objectives and end points of the study were to evaluate the safety of hu3S193 in patients with advanced epithelial cancers expressing the Le y antigen; determine the pharmacokinetics, tissue distribution, and imaging characteristics of i.v. administered ^{111}In -hu3S193; determine the patient's immune response to hu3S193; and to document observed tumor responses. The protocol was approved by the Human Research and Ethics Committee of the Austin Hospital prior to study commencement. All patients gave written informed consent before study entry. The phase I trial was conducted via a Therapeutic Goods Administration clinical trial notification scheme and under a Food and Drug Administration Investigational New Drug Application.

Eligibility criteria included advanced epithelial cancer in patients who had failed at least one line of chemotherapy and/or hormonal therapy but had not received more than three lines of therapy for metastatic disease; measurable or evaluable disease histologically proven to express Le y antigen; Karnofsky performance status $\geq 70\%$ with no serious co-morbidity; expected survival of ≥ 4 months; adequate marrow, renal, and hepatic function; left ventricular ejection fraction $>50\%$; and no concurrent immunosuppressive therapy. Following immunohistochemical assessment of archived tumor samples for Le y expression, tissue sections were graded as - (negative), + (weak), ++ (moderate), and +++ (strong). Tumors were defined as Le y positive if $>50\%$ of cells were weakly stained, or $>30\%$ were moderate to strongly stained.

Hu3S193 was administered weekly $\times 4$ doses at one of four dose levels (5, 10, 20, or 40 mg/m^2) by i.v. infusion over a period of 60 min. The first dose of hu3S193 was trace radiolabeled with Indium-111 (^{111}In ; 200–280 MBq, 5–7 mCi) to assess biodistribution and targeting to tumor. Tumor evaluation was done before treatment and 2 weeks after the fourth dose. After one cycle, patients showing evidence of objective tumor response were offered further cycles at the same dose level for up to a further 12 months.

Dose escalation criteria

The first patient at each dose level was observed for 4 weeks before enrollment of any additional patients. If no dose-limiting toxicity (DLT) was observed in any of the first three patients within 4 weeks of the first infusion of hu3S193, three patients were then entered on the next highest dosage tier. If one patient in any cohort of three patients experienced a DLT within 4 weeks from the first dose, an additional three patients (maximum of six) were entered at that dosage level. If no more than one patient out of six in any dose level experienced grade ≥ 3 toxicity, subsequent patients were entered at the next dosage tier.

DLT was defined as grade 3 nonhematologic toxicity, or grade 4 hematologic toxicity as defined by the National Cancer Institute

Common Toxicity Criteria for Adverse Events version 3.0 (CTCAE v3.0). Maximum tolerated dose was defined as the hu3S193 dose below that where two or more patients out of six experienced DLT.

Radiolabeling of Hu3S193

The antibody hu3S193 was labeled with ^{111}In (MDS Nordion) via the bifunctional metal ion chelate CHX-A'-diethylenetriaminepenta-acetic acid according to methods described previously (29, 30).

Gamma camera imaging

Whole-body images of ^{111}In -hu3S193 biodistribution were obtained in all patients on day 0 after infusion of ^{111}In -hu3S193 and on at least three further occasions up to day 7 following infusion. Single-photon emission computed tomography images of a region of the body with known tumor were also obtained on at least one occasion during this period. All gamma camera images were acquired on dual-headed gamma cameras (Trionix Research Laboratories or Picker International).

Pharmacokinetics

Blood samples were collected for pharmacokinetics to be analyzed by ELISA for each hu3S193 infusion and by ^{111}In measurement following the first infusion.

Radiolabeled hu3S193. Serum samples were aliquoted in duplicate and counted in a gamma scintillation counter (Packard Instruments), along with appropriate ^{111}In standards. The results of the serum were expressed as % injected dose per liter (% ID/L).

Pharmacokinetic calculations were done of serum data using a curve fitting program (WinNonlin, Pharsight Co.). A two-compartment model was fitted to individual labeled infusions for each patient using unweighted nonlinear least squares to calculate pharmacokinetic variables of $T_{1/2\alpha}$ and $T_{1/2\beta}$, V_1 , area under the curve, and clearance.

ELISA. Measurement of patient serum hu3S193 protein levels following each infusion was done in triplicate using a validated ELISA assay with a 3.0 ng/mL limit of detection, as previously described (31). A two-compartment model (ADVAN3) was fitted to the pharmacokinetic data from all patients using NONMEM (University of California, San Francisco, CA). Peak and trough serum hu3S193 levels (C_{min} , C_{max}), clearance, and area under the curve were calculated for each infusion.

Tumor and organ dosimetry of ^{111}In -hu3S193

Regions of interest were defined for suitable tumors at each time point on ^{111}In -hu3S193 image data sets, corrected for background and attenuation, and dosimetry calculation was performed to derive the concentration of ^{111}In -hu3S193 in tumor per gram (32–34). This was converted to milligram hu3S193 per gram tumor tissue based on the injected milligram hu3S193 protein dose. A similar approach was used to calculate uptake in stomach wall at various time points post infusion.

Immune effector function of hu3S193 *in vivo*

Serum samples were collected from six patients on day 28 (± 1 day) following their final infusion of hu3S193 (three patients each at the 20 and 40 mg/m^2 dose levels). The serum samples were heated to 56°C for 30 min to destroy any endogenous complement activity. The samples were then used as a source of hu3S193 antibody in CDC and ADCC assays, with control complement and peripheral blood mononuclear cells used in assays (35, 36). Controls of healthy donor serum added to equivalent levels of hu3S193 and isotype control huA33 (37) were treated and analyzed concurrently with the test samples.

Hu3S193-mediated CDC and ADCC activity in patient serum was measured in triplicate with a 4-h ^{51}Cr release assay based upon previously published methods (35, 36).

Human anti-human antibody

Blood samples for human anti-human antibody (HAHA) assessment were taken before each hu3S193 infusion, then at week 6 and at 30 days

after last hu3S193 infusion, and were analyzed by surface plasmon resonance technology using a BIAcore2000 instrument as previously described (38).

Results

Patients. Fifteen patients with a mean age of 53 years (range, 42-76 years) completed the trial (Table 1). Primary tumor sites, prior therapy history, and sites of disease at study entry are also shown in Table 1. All 15 patients had Le^y-positive tumors on screening and fulfilled all inclusion criteria.

Adverse events and HAHA. Adverse events related to hu3S193 are listed in Tables 2 and 3. Overall, hu3S193 was safe and well tolerated at all dose levels, with generally predictable and manageable toxicities being observed. Transient grade 1 to 2 nausea and vomiting was observed following infusion of hu3S193 at the 40 mg/m² dose level only. These symptoms did not occur following all infusions and were self-limiting. The maximum tolerated dose was not reached. One episode of DLT was observed, with asymptomatic grade 3 alkaline phosphatase liver enzyme increase in a patient (patient 05) with extensive liver metastases (baseline grade 2 alkaline phosphatase elevation at study entry). This event was associated with right upper quadrant pain. Elevated alkaline phosphatase resolved to baseline levels following cessation of hu3S193

infusions. No additional DLTs were observed in this expanded cohort nor in higher dose levels. Liver enzyme abnormalities attributable to study drug were not observed in any other patient.

One patient (patient 04, 10 mg/m²) experienced grade 1 chest wall paresthesia and axillary swelling at sites of known disease that was related to the second and fourth hu3S193 infusions.

Grade 1 and 2 asymptomatic elevations in complement levels were also noted in 10 patients. In all but three patients this had resolved at last follow-up. There were no significant changes observed in blood counts, serum electrolytes, or creatinine in any patient. No episodes of delayed toxicity were observed in the follow-up of the 15 patients.

No HAHA was detected in serum. A transient episode of localized urticaria was experienced by one patient (patient 14, 40 mg/m²) with no BIAcore evidence of HAHA. This occurred during the first infusion of hu3S193, resolved spontaneously, and did not recur with subsequent infusions.

Radiolabeling of hu3S193. There were a total of 17 infusions of ¹¹¹In-hu3S193 administered during the trial. The mean ± SD immunoreactivity of ¹¹¹In-hu3S193 was measured to be 60.1 ± 8.9%.

Biodistribution of hu3S193. The pattern of ¹¹¹In-hu3S193 biodistribution in patients at the 5, 10, and 20 mg/m² dose

Table 1. Patient characteristics

Patient no.	Dose level (mg/m ²)	Age at study entry (y)	Sex	KPS at study entry (%)	Site of primary tumor	Prior therapies	Regional disease sites at study entry	Tumor response to hu3S193
1	5	41	F	70	Breast	Surgery, radiotherapy, hormonal (3 lines)	Lung	SD after cycle 1
2	5	51	F	80	Breast	Surgery, radiotherapy, chemotherapy (2 lines)	Lymph nodes, s.c. and cutaneous	PD after cycle 2
3	5	66	M	100	Colon	Surgery, chemotherapy (1 line)	Liver	PD after cycle 1
4	10	45	F	8	Breast	Surgery, radiotherapy, chemotherapy (4 lines)	Cutaneous and lymph nodes	PD after cycle 2
5	10	43	F	80	Breast	Surgery, radiotherapy, chemotherapy (4 lines)	Lymph nodes, liver, lung	PD after cycle 1
6	10	45	M	100	Colon	Surgery, radiotherapy, chemotherapy (3 lines)	Lung	SD after cycle 1
7	10	70	M	80	Colon	Surgery, radiotherapy, chemotherapy (2 lines)	Liver and spleen	PD after cycle 1
8	10	65	M	90	Colon	Surgery, chemotherapy (1 line)	Liver	PD after cycle 1
9	10	65	M	80	Rectum	Surgery, radiotherapy, chemotherapy (3 lines)	Lung and rectum lymph nodes	PD after cycle 1
10	20	47	F	80	Colon	Surgery, chemotherapy (1 line)	Liver lymph nodes	PD after cycle 1
11	20	76	M	70	Rectum	Radiotherapy, chemotherapy (1 line)	Liver, lung	PD after cycle 1
12	20	46	F	80	Colon	Surgery, chemotherapy (2 lines)	Liver	PD after cycle 1
13	40	49	F	80	Breast	Surgery radiotherapy, chemotherapy (2 lines)	Breast, lymph nodes s.c.	PD after cycle 1
14	40	42	F	100	Breast	Surgery, radiotherapy, chemotherapy (2 lines)	Bone	SD after cycle 1
15	40	42	M	100	NSCLC	Chemotherapy (2 lines)	Lymph nodes	PD after cycle 1

Abbreviations: M, male; F, female; KPS, Karnofsky performance status; NSCLC, non-small-cell lung cancer; PD, progressive disease; SD, stable disease; s.c., subcutaneous.

Table 2. Occurrence of adverse events related to hu3S193

Adverse event	Dose level (mg/m ²)*				Total number of episodes of each event
	5	10	20	40	
Pruritus	0	0	0	1	1
Urticaria	0	0	0	1	1
Dyspepsia	0	0	0	2	2
Nausea	0	0	0	13	13
Vomiting	0	0	0	9	9
ALP: elevated †	0	1	0	0	1
ALT: elevated	0	2	0	0	2
AST: elevated	0	1	0	0	1
Complement C3: elevated	0	4	1	2	7
Complement C4: elevated	0	1	0	0	1
Complement THC: elevated	2	1	0	1	4
Swelling: left axilla	0	1	0	0	1
Paraesthesia: chest wall	0	1	0	0	1
Pain: abdominal RUQ	0	2	0	0	2
Pain: chest wall	0	1	0	0	1
Total	2	15	1	29	47

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; THC, total hemolytic complement.

*Numbers represent number of episodes of any event at each dose level.

†DLT (patient 05).

levels was consistent with blood pool activity, which cleared gradually with time. Some minor gut uptake was observed, consistent with gut excretion of free ¹¹¹In. At these dose levels, no other normal tissue uptake was observed (Fig. 1). For the two patients receiving infusions of ¹¹¹In-hu3S193 in an additional cycle, no change in biodistribution pattern was observed in either patient.

At the 40 mg/m² dose level, in two patients (patients 13 and 14), some stomach and bowel activity was observed after infusion (day 0 and day 1), which rapidly resolved (Fig. 2). This was associated with infusion related symptoms of nausea and vomiting. Abnormal uptake in stomach or bowel was not seen in patient 15. No other normal tissue uptake was seen at this dose level.

Excellent uptake of ¹¹¹In-hu3S193 was observed in tumor sites at all dose levels, with metastatic lesions greater than 2.0 cm visualized in lung, liver, lymph nodes, and bone. Subcutaneous lesions and small metastatic disease in the omentum less than 1.5 cm in size were also visualized (patients 4 and 12).

Pharmacokinetics. The results of pharmacokinetic analysis for ¹¹¹In-hu3S193 are shown in Table 4. The final mean ± SD

pharmacokinetic analysis for unlabeled hu3S193 (based on ELISA) was T_{1/2α} = 10.95 ± 0.63 h, T_{1/2β} = 162.41 ± 35.88 h, clearance = 36.84 ± 12.16 mL/h, and V₁ = 3.93 ± 0.88. No significant differences or trends were observed between dose level and T_{1/2α}, T_{1/2β}, volume of distribution, or clearance. As expected, linear relationships were observed for area under the curve, C_{max}, and C_{min} with dose level (data not shown). Following the first infusion, peak serum hu3S193 concentrations ranged from 1.9 ± 0.55 µg/mL (5 mg/m² dose level) to 25.07 ± 2.95 mg/mL (40 mg/m² dose level; see Supplementary Information).

Tumor and stomach dosimetry of ¹¹¹In-hu3S193. Tumor dosimetry analysis was completed for 9 of 15 patients. Tumor dosimetry was not done on patients 1, 3, 5, 6, 13, and 14 because tumor was not easily distinguishable on static gamma camera images due to small lesion size. The calculated peak uptake of hu3S193 in tumor ranged from 1.2 to 6.3 µg/g (mean ± SD µg/g: 5 mg/m², 1.50 ± 0; 10 mg/m², 3.25 ± 1.96; 20 mg/m², 1.93 ± 0.36; 40 mg/m², 5.43 ± 0).

Stomach uptake was maximal at day 0 (immediately after infusion) and increased with dose level (mean ± SD µg/mg: 5 mg/m², 0.0062 ± 0.0054; 10 mg/m², 0.025 ± 0.016;

Table 3. Grade of adverse events related to hu3S193

Dose level (mg/m ²)	Maximum CTC grade toxicity*			
	1, Mild	2, Moderate	3, Severe	4, Life-threatening
5	2	0	0	0
10	11	3	1	0
20	1	0	0	0
40	23	6	0	0
Overall	37	9	1	0

*Number of patients.

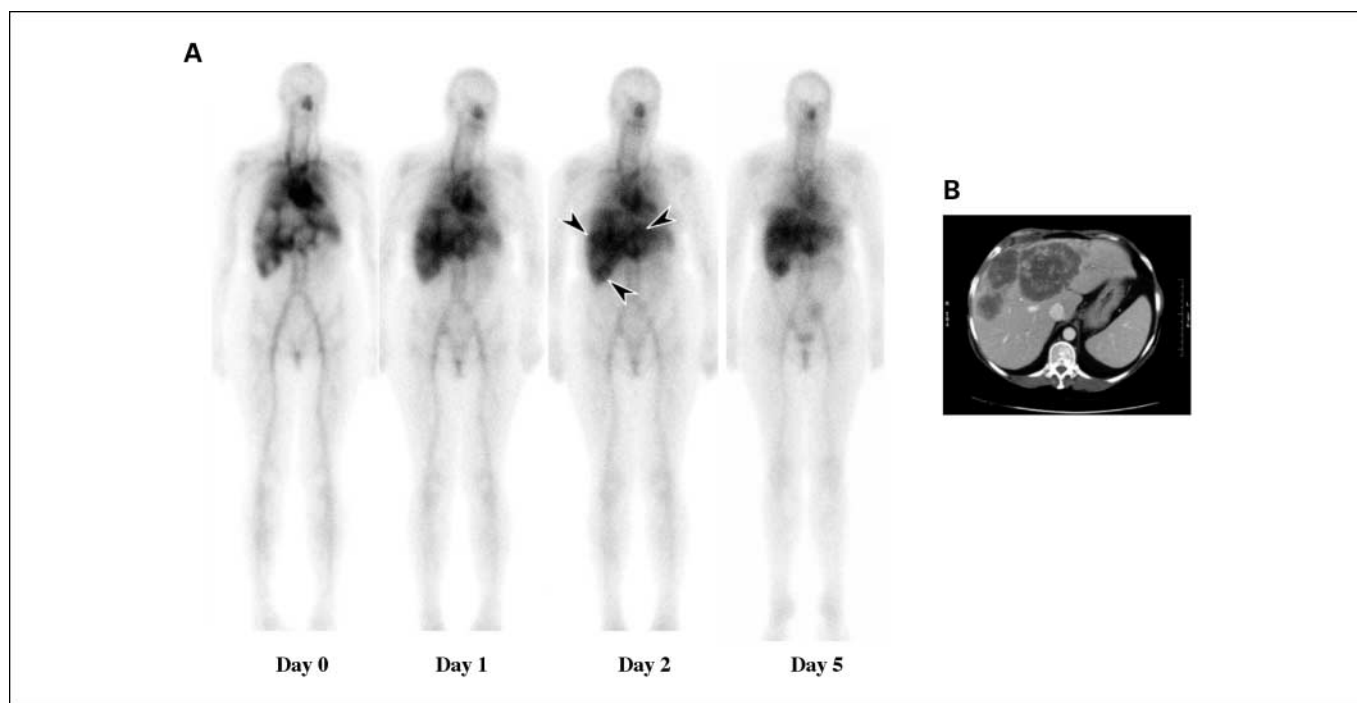


Fig. 1. Biodistribution of ^{111}In -hu3S193 in patient 10 (20 mg/m² dose level). *A.* anterior whole-body gamma camera images over 5 d after infusion. Initial blood pool activity (day 0) is seen, with gradual clearance with time. Uptake of ^{111}In -hu3S193 in metastatic colon carcinoma in the liver is seen by day 2 (*arrows*) and increases with time to day 5. *B.* computed tomography scan of the upper abdomen showing metastatic colon carcinoma.

20 mg/m², 0.033 ± 0.021 ; 40 mg/m², 0.18 ± 0.15). At the 40 mg/m² dose level, activity within the stomach was observed in two patients (Fig. 2), which resulted in a probable overestimate of concentration of hu3S193 in stomach wall in these patients.

Retention of immune effector function of hu3S193 in vivo. The CDC and ADCC results showed retention of hu3S193 immune effector function in patient sera for up to 1 week

following administration, with ADCC and CDC levels equivalent to that achieved with fresh hu3S193 added to heat-inactivated healthy donor serum (see Supplementary Information).

Tumor responses. Staging at the completion of cycle 1 showed 11 patients with progressive disease and 4 patients with stable disease (Table 1). Patients 01 and 03 successfully

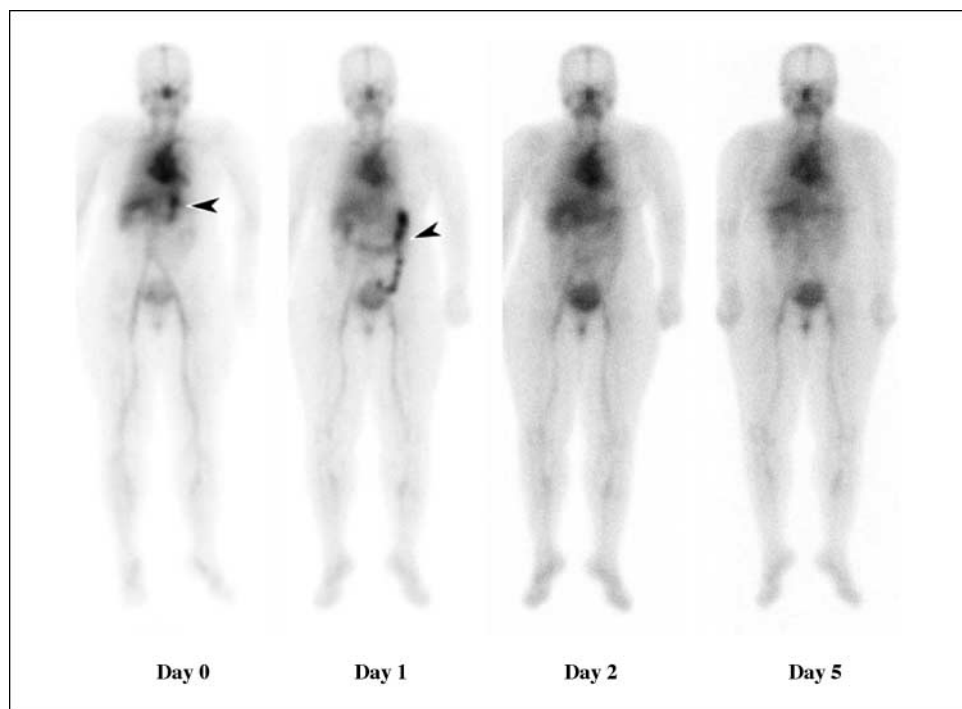


Fig. 2. Biodistribution of ^{111}In -hu3S193 in patient 14 (40 mg/m² dose level). Anterior whole-body gamma camera images over 7 d after infusion. On day 0, blood pool activity is seen, and some activity in the stomach is also evident (*arrow*). This patient had symptoms of nausea following the infusion and before imaging. By day 1, stomach activity is no longer seen, and activity in the lumen of the large intestine is evident (*arrow*). This has completely cleared by day 2, and no further gastrointestinal activity is seen at day 5.

Table 4. Pharmacokinetic parameters of $^{111}\text{In-CHX-A''-DTPA-hu3S193}$

Dose level (mg/m ²)	T ^{1/2} α (h), mean (SD)	T ^{1/2} β (h), mean (SD)	V ₁ (mL), mean (SD)	Clearance (mL/h), mean (SD)	AUC (h·μg/mL), mean (SD)
5	3.45 (1.08)	166.9 (57.61)	3,657.67 (671.77)	32.01 (14.18)	305.18 (95.09)
10	5.40 (4.86)	166.67 (54.12)	3,586.42 (388.19)	23.93 (6.86)	803.69 (266.91)
20	11.98 (7.56)	221.23 (83.07)	2,711.2 (486.27)	15.87 (7.27)	2,220.88 (773.17)
40	6.66 (5.87)	226.67 (60.02)	2,842.51 (746.03)	14.69 (3.54)	4,964.11 (412.56)
All	6.58 (5.53)	189.63 (62.17)	3,276.85 (642.43)	22.09 (9.87)	

Abbreviation: AUC, area under the curve.

completed a second cycle of treatment. At restaging, both had developed progressive disease. Patient 05 (10 mg/m² dose level) did, however, have objective evidence of reduction in size of a clinically palpable left cervical lymph node (14 cm² to 1.5 cm²) after one cycle of hu3S193 and had stable disease at last follow-up.

Discussion

This study represents the first reported demonstration of the biodistribution and targeting of a humanized anti-Le^y antibody in patients with epithelial cancers. At doses up to 40 mg/m² given every week, hu3S193 was well tolerated, and maximum tolerated dose was not reached. The biodistribution of hu3S193 in all patients showed gradual clearance of blood pool activity and no consistent normal tissue uptake of $^{111}\text{In-hu3S193}$. Excellent tumor uptake of hu3S193 was also evident, including lung, liver, lymph node, soft tissue and bone metastases, indicating the selectivity of hu3S193 for epithelial tumors known to express the Le^y antigen. Importantly, no HAHA responses to hu3S193 were observed in any patient entered into the study.

The principal toxicity of hu3S193 was grade 1 to 2 nausea and vomiting at the 40 mg/m² dose level, which was self-limiting. This was associated with (in two patients) some stomach activity on day 0, which cleared by 24 h, and bowel activity subsequently disappeared after 1 to 2 days (Fig. 2). The timing of these symptoms, together with the biodistribution evident on gamma camera images, suggests that the peak concentration of hu3S193 in blood at the 40 mg/m² dose level immediately after infusion (>20 μg/mL) may induce a self-limiting inflammatory process in the gastric mucosa. This observation is consistent with the known expression of Le^y antigen in gastric mucosa (1–5). The activity in the stomach (Fig. 2) in the two patients with gastrointestinal symptoms may represent shed $^{111}\text{In-hu3S193}$, or minor blood loss, which traveled within the lumen of the bowel over subsequent days. Calculation of concentration of $^{111}\text{In-hu3S193}$ in stomach wall showed gradual increase with dose, although results were confounded at the 40 mg/m² dose level due to activity within the stomach lumen. In the two patients with symptoms related to the first infusion of hu3S193, no significant drop in blood counts was observed. This observation of gastric symptoms is similar to that reported with IG311 (15) and with BR96-doxorubicin (SGN-15; refs. 18–21), although this is the first study to identify the temporal relationship of symptoms to the biodistribution of anti-Le^y antibody to stomach and gut.

Pharmacokinetic analysis revealed hu3S193 to have a biphasic serum clearance and a long terminal half-life of greater than 1 week. Importantly, no saturable normal tissue compartment was identified, and serum levels increased proportionally with dose. Trough levels of hu3S193 of over 1 μg/mL were seen at dose levels of 10 mg/m² and higher. This concentration achieved >50% killing of tumor cells in *in vitro* preclinical studies (22) and indicates that biologically significant concentrations could be achieved at the dose levels studied. These results are quite different from humanized antibodies against antigens expressed at high levels in normal tissue (e.g., CD20, epidermal growth factor receptor, ErbB2) where large loading doses are required to achieve saturable pharmacokinetics (39, 40). Quantitative image analysis of tumor uptake at 6 to 7 days after infusion showed concentrations of hu3S193 of >1 μg/g tumor in all patients and increasing accumulation of hu3S193 with dose level. The use of ^{111}In to radiolabel hu3S193 enabled accurate quantitative assessment of tumor uptake and retention of hu3S193, whereas radiohalides (e.g., ^{131}I) would have undergone rapid dehalogenation in the tumor, and subsequent extrusion from the tumor cell (22–24). Hu3S193 also retained potent immune effector function *in vivo* (both CDC and ADCC). The high concentration of hu3S193 in tumor would allow the continued exposure of tumor cell surface to hu3S193 and immune mediators (complement and effector cells) resulting in optimal conditions for cell killing. These data confirm the ability of hu3S193 to selectively target tumors in patients at concentrations suitable for potent immunologic effect.

Although no objective tumor responses were seen, the schedule of dosing was for only 4 weeks, which may have limited the potential for the effects of hu3S193 to be measured. Interestingly, some biological effect of hu3S193 was observed, including clinical shrinkage of a lymph node in one patient, and another patient experienced left chest wall paresthesia and swelling associated with the second and fourth hu3S193 infusion. The DLT observed (elevated alkaline phosphatase) was also potentially related to the enlargement of lymph nodes near the porta hepatis of the patient, further suggesting a biological effect of hu3S193.

Although other antibodies targeting the Le^y antigen have been studied in the clinic, hu3S193 has a number of important properties that make it highly attractive as a therapeutic. First, this trial has clearly showed that hu3S193 selectively targets Le^y-expressing tumors at high concentrations and with retention of immune effector function *in vivo*. It is well tolerated, and the lack of immunogenicity is in contrast to SGN-15 (18). The favorable pharmacokinetics of hu3S193 and high tumor

concentrations achieved at the dose levels studied in this trial support the use of hu3S193 for immune therapy of solid tumors, alone or in combination with other treatments. Hu3S193 has also been shown to have improved efficacy when radiolabeled and combined with chemotherapy or linked to a drug in preclinical models (25, 26, 28). Collectively, these results, therefore, indicate the potential for hu3S193 treatment

of metastatic Le^y-expressing cancers, and further phase I/II trials aimed at optimizing dosage and scheduling of hu3S193 are ongoing.

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References

- Hakomori S. Tumor-associated carbohydrate antigens. *Annu Rev Immunol* 1984;2:103–26.
- Sakamoto J, Furukawa K, Cordon-Cardo C, et al. Expression of Lewisia, Lewisb, X, and Y blood group antigens in human colonic tumors and normal tissue and in human tumor-derived cell lines. *Cancer Res* 1986;46:1553–61.
- Miyake M, Taki T, Hitomi S, Hakomori S. Correlation of expression of H/Le^y/Le^b antigens with survival in patients with carcinoma of the lung. *N Eng J Med* 1992;327:14–8.
- Kitamura K, Stockert E, Garin-Chesa P, et al. Specificity analysis of blood group Lewis-y (Le(y)) antibodies generated against synthetic and natural Le(y) determinants. *Proc Natl Acad Sci U S A* 1994;91:12957–61.
- Zhang S, Zhang HS, Cordon-Cardo C, et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: II. Blood group-related antigens. *Int J Cancer* 1997;73:50–6.
- Yuriev E, Farrugia W, Scott AM, Ramsland P. Three-dimensional structures of carbohydrate determinants of Lewis system antigens: implications for effective antibody targeting of cancer. *Immunol Cell Biol* 2005;83:709–17.
- Hellstrom I, Garrigues HF, Garrigues U, Hellstrom KE. Highly tumor-reactive, internalising, mouse monoclonal antibodies to Le^y related cell surface antigens. *Cancer Res* 1990;50:2183–90.
- Yin BW, Finstad CL, Kitamura K, et al. Serological and immunochemical analysis of Lewis y (Ley) blood group antigen expression in epithelial ovarian cancer. *Int J Cancer* 1996;65:406–12.
- Furukawa K, Welt S, Yin BW, et al. Analysis of the specificity of eleven mouse monoclonal antibodies reactive with type 2 blood group determinants. *Mol Immunol* 1990;27:723–32.
- Steplewski Z, Lubeck MD, Scholtz D, Loibner H, Smith JM, Koprowski H. Tumor cell lysis and tumor growth inhibition by the isotype variants of Mab BR55–2 directed against Y oligosaccharide. *In Vivo* 1991;5:79–83.
- Theodoulou M, Gilewski TA, Welt S, et al. Anti-lewis Y (Ley) monoclonal antibody (mAb) BR55–2 (IgG2a) in patients with advanced breast cancer [abstract]. *Proc Am Soc Clin Oncol* 1994;13:A974.
- Schlimok G, Fackler-Schwalbe I, Pantel K, Loibner H, Riethmüller G. Monoclonal Lewis Y antibody depletes metastatic breast carcinoma cells from bone marrow [abstract]. *Proc Am Soc Clin Oncol* 1993;12:289.
- Pai-Scherf LH, Carrasquillo JA, Paik C, et al. Imaging and phase I study of ¹¹¹In- and ⁹⁰Y-labeled Anti-LewisY monoclonal antibody B3. *Clin Cancer Res* 2000;6:1720–30.
- Pai LH, Wittes R, Setser A, Willingham WC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. *Nat Med* 1996;2:350–3.
- Orozio DV, Aulmann C, Eller N, et al. Results from a phase I clinical trial with IGN311, a fully humanized IgG1 antibody against Lewis Y in patients with solid tumors [abstract]. *Proc Am Soc Clin Oncol* 2004;22:2624.
- Schreiber GJ, Hellstrom KE, Hellstrom I. An unmodified anticarcinoma antibody BR96 localised to and inhibits the outgrowth of human tumors in nude mice. *Cancer Res* 1992;52:3262–6.
- Trail PA, Willner D, Lach SJ, et al. Cure of xenografted human carcinomas by BR96-doxorubicin immunconjugates. *Science* 1993;261:212–5.
- Saleh MN, Sugarman S, Murray J, et al. Phase I trial of the anti-Lewis Y drug immunconjugate BR96-doxorubicin in patients with Lewis Y-expressing epithelial tumors. *J Clin Oncol* 2000;18:2282–92.
- Tolcher AW, Sugarman S, Gelmon KA, et al. Randomized phase II study of BR96-doxorubicin conjugate in patients with metastatic breast cancer. *J Clin Oncol* 1999;17:478–84.
- Ajani JA, Kelsen DP, Haller D, Hargraves K, Healey D. A multi-institutional phase II study of BMS-182248-01 (BR96-doxorubicin conjugate) administered every 21 days in patients with advanced gastric adenocarcinoma. *Cancer J* 2000;6:78–81.
- Ross HJ, Rudin CM, Hart LL, et al. Randomized phase II trial of SGN-15 (BR96-doxorubicin immunconjugate) with docetaxel in patients with advanced or metastatic non-small cell lung cancer [abstract]. *Proc Am Soc Clin Oncol* 2004;22:7039.
- Scott AM, Geleick D, Rubira M, et al. Construction, production, and characterization of humanized anti-Lewis Y monoclonal antibody 3S193 for targeted immunotherapy of solid tumors. *Cancer Res* 2000;60:3254–61.
- Clarke K, Lee FT, Brechbiel MW, Smyth FE, Old LJ, Scott AM. *In vivo* biodistribution of a humanized anti-Lewis Y monoclonal antibody (hu3S193) in MCF-7 xenografted BALB/c nude mice. *Cancer Res* 2000;60:4804–11.
- Clarke K, Lee FT, Brechbiel MW, Smyth FE, Old LJ, Scott AM. Therapeutic efficacy of anti-Lewis^y humanised 3S193 (Hu3S193) radioimmunotherapy in a breast cancer model: enhanced activity when combined with Taxol chemotherapy. *Clin Cancer Res* 2000;6:3621–8.
- Lee FT, Mountain AJ, Kelly M, et al. Enhanced efficacy of radioimmunotherapy with ⁹⁰Y-CHX-A'-DTPA-hu3S193 by inhibition of epidermal growth factor receptor (EGFR) signalling with EGFR tyrosine kinase inhibitor AG1478. *Clin Cancer Res* 2005;11:7080–6s.
- Kelly MP, Lee FT, Smyth FE, Brechbiel MW, Scott AM. Enhanced efficacy of yttrium-90 radiolabelled anti-Lewis-Y humanised monoclonal antibody hu3S193 and paclitaxel combined modality radioimmunotherapy in a breast cancer model. *J Nucl Med* 2006;47:716–25.
- Ramsland PA, Farrugia W, Bradford TM, Hogarth PM, Scott AM. Structural convergence of antibody binding sites for carbohydrate determinants in Lewis^y tumour antigens. *J Mol Biol* 2004;340:809–18.
- Boghaert ER, Sridharan L, Armellino DC, et al. Antibody-targeted chemotherapy with the calicheamicin conjugate hu3S193-N-acetyl gamma calicheamicin dimethyl hydrazide targets Lewisy and eliminates Lewisy-positive human carcinoma cells and xenografts. *Clin Cancer Res* 2004;10:4538–49.
- Nikula TK, Curcio MJ, Brechbiel MW, et al. A rapid, single vessel method for preparation of clinical grade ligand conjugated monoclonal antibodies. *Nucl Med Biol* 1995;22:387–90.
- Scott AM, Lee FT, Hopkins W, et al. Specific targeting, biodistribution and lack of immunogenicity of chimeric anti-GD3 monoclonal antibody KM871 in patients with metastatic melanoma: results of a phase I trial. *J Clin Oncol* 2001;19:3976–87.
- Liu Z, Panousis C, Smyth FE, et al. Generation of anti-idiotypic antibodies for application in clinical immunotherapy laboratory analyses. *Hybrid Hybridomics* 2003;22:219–28.
- Liu A, Williams LE, Raubitschek AA. A CT assisted method for absolute quantitation of internal radioactivity. *Med Phys* 1996;23:1919–28.
- Loevinger R, Budinger TF, Watson EE. *MIRD primer for absorbed dose calculations*. New York: The Society of Nuclear Medicine; 1991.
- Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: The second-generation personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 2005;46:1023–7.
- Nelson DL, Kurman CC, Serbousek CC. 51-Cr release assay of antibody-dependent cell-mediated cytotoxicity (ADCC). Current protocols in immunology. In: Coligan JE, Kruisbeek AM, Margulies DD, Shevach EM, Strober W, editors. New York: Greene Publishing Wiley Interscience; 1991. p. 7.27.1.
- Scott AM, Liu Z, Murone C, et al. Immunological effects of chimeric anti-GD3 monoclonal antibody KM871 in patients with metastatic melanoma. *Cancer Immunity* [serial on the Internet]. 2005; 5:3. Available from: <http://www.cancerimmunity.org/v5p3/041221.htm>.
- Scott AM, Lee FT, Jones R, et al. Phase I biopsys-based study of humanised monoclonal antibody A33 in patients with colorectal carcinoma. *Clin Cancer Res* 2005;11:4810–7.
- Ritter G, Cohen LS, Williams C, Jr., Richards EC, Old LJ, Welt S. Serological analysis of human anti-human antibody responses in colon cancer patients treated with repeated doses of humanized monoclonal antibody A33. *Cancer Res* 2001;61:6851–9.
- Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997;90:2188–95.
- Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol* 2005;23:2445–59.