FULL PAPER

BJC

British Journal of Cancer (2013) 109, 934–942 | doi: 10.1038/bjc.2013.376

Keywords: pretargeting; bispecific antibodies; CEA; radionuclide imaging; radionuclide therapy; colorectal cancer

Development of an imaging-guided CEA-pretargeted radionuclide treatment of advanced colorectal cancer: first clinical results

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Background: Radiolabelled antibody targeting of cancer is limited by slow blood clearance. Pretargeting with a non-radiolabelled bispecific monoclonal antibody (bsMAb) followed by a rapidly clearing radiolabelled hapten peptide improves tumour localisation. The primary goals of this first pretargeting study in patients with the anti-CEACAM5 × anti-hapten (HSG) bsMAb, TF2, and the radiolabelled hapten-peptide, IMP288, were to assess optimal pretargeting conditions and safety in patients with metastatic colorectal cancer (CRC).

Methods: Different dose schedules were studied in four cohorts of five patients: (1) shortening the interval between the bsMAb and peptide administration (5 days *vs* 1 day), (2) escalating the TF2 dose (from 75 to 150 mg), and (3) reducing the peptide dose (from 100 to 25 μg). After confirmation of tumour targeting by ^{111}In-IMP288, patients were treated with a bsMAb/^{177}Lu-IMP288 cycle.

Results: Rapid and selective tumour targeting of the radiolabelled peptide was visualised within 1 h, with high tumour-to-tissue ratios (> 20 at 24 h). Improved tumour targeting was achieved with a 1-day interval between the administration of the bsMAb and the peptide and with the 25-μg peptide dose. High ^{177}Lu-IMP288 doses (2.5–7.4 GBq) were well tolerated, with some manageable TF2 infusion reactions, and transient grades 3–4 thrombocytopaenia in 10% of the patients who received ^{177}Lu-IMP288.

Conclusion: This phase I study demonstrates for the first time that pretargeting with bsMAb TF2 and radiolabelled IMP288 in patients with CEA-expressing CRC is feasible and safe. With this pretargeting method, tumours are specifically and rapidly targeted.

Tumour targeting with monoclonal antibodies is an attractive approach for selective cancer therapy. For metastatic colorectal cancer (CRC), the anti-vascular growth factor receptor antibody, bevacizumab, and the anti-epidermal growth factor receptor antibodies, cetuximab or panitumumab, can improve patient outcome when combined with chemotherapy (Hurwitz *et al*, 2004; Giantonio *et al*, 2007; Amado *et al*, 2008; Saltz *et al*, 2008; Bokemeyer *et al*, 2009; Tol *et al*, 2009; Van Cutsem *et al*, 2009). Antibodies conjugated with cytotoxic agents, such as drugs or radionuclides, have shown promising results in several indications (Govindan and Goldenberg, 2010; Sharkey and Goldenberg, 2011). Radiolabelled antibodies have proven effective in patients with non-Hodgkin lymphoma (Wiseman *et al*, 2002; Witzig *et al*, 2002a,b; Gordon *et al*, 2004), but successful

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Received 12 February 2013; revised 18 June 2013; accepted 24 June 2013; published online 16 July 2013

adaptation of radioimmunotherapy (RIT) in solid tumours has been challenging (Liersch *et al*, 2005; Sharkey and Goldenberg, 2011).

The slow blood clearance and delayed tumour uptake of directly radiolabelled antibodies cause continuous radiation exposure to the bone marrow and a high background signal. Pretargeting techniques were developed to overcome these limitations. With pretargeting, a non-radiolabelled humanised bispecific monoclonal antibody (bsMAb) is administered first intravenously. After the bsMAb localises in the tumour and clears from the circulation, a radiolabelled hapten peptide is given that is rapidly trapped in the tumour by the bsMAb, while the remainder clears from the blood very quickly, being eliminated via the kidneys. Pretargeting reduces the radiation exposure to radiosensitive normal tissues, such as bone marrow, as well as other tissues (Reardan *et al*, 1985; Chang *et al*, 2002; Boerman *et al*, 2003; Sharkey *et al*, 2005).

In this first-in-man phase I study, we investigated pretargeting with the bsMAb, TF2, for targeting CRC. TF2 is a humanised tri-Fab molecule (Rossi *et al*, 2006), consisting of two anti-CEACAM5 Fab fragments and another Fab fragment with affinity for the hapten, histamine-succinyl-glycine (HSG). IMP288 is a hapten peptide that contains two HSG moieties to preserve the affinity enhancement properties for improved uptake and retention (Le Doussal *et al*, 1989; Karacay *et al*, 2000), and another moiety capable of stable binding of a radionuclide, in this case 1,4,7, 10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) for binding ^{90}Y, ^{177}Lu, and ^{111}In (Rossi *et al*, 2006; Goldenberg *et al*, 2008; Schoffelen *et al*, 2010a). The rapid and specific targeting of human tumour xenografts and the therapeutic potential of pretargeted, radiolabelled hapten-peptides were reported previously (Sharkey *et al*, 2008; Schoffelen *et al*, 2010b). The primary goals of this trial were to evaluate several pretargeting conditions and to assess the safety of pretargeting with TF2 and ^{177}Lu-labelled IMP288 in patients with metastatic CRC for whom no standard treatment was available.

METHODS

Patient eligibility. Patients $\geqslant$18 years of age with progressive metastatic CRC for whom no standard treatment was available were enrolled. Eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant$1, no previous therapies within 4 weeks (bevacizumab within 8 weeks), and adequate haematopoiesis (absolute neutrophil count $\geqslant 1.5 \times 10^9$ per litre; platelets $\geqslant 150 \times 10^9$ per litre without transfusion during the previous month; haemoglobin $\geqslant$5.6 mmol l^{-1}), and with acceptable hepatic (total bilirubin $\leqslant 2 \times$ upper limit of normal (ULN), aspartate transaminase (AST)/alanine transaminase (ALT) $\leqslant 3 \times$ ULN) and renal function (serum creatinine $\leqslant 2 \times$ ULN, Cockcroft clearance $>$ 50 ml min^{-1}). Evidence of CEA expression by tissue staining ($>$20% of the tumour cells CEA positive) or elevated plasma levels was required. Patients with a life expectancy of $<$6 months, known brain metastases, or cardiac disease with New York Heart Association classification of III or IV, were excluded.

The regional ethics review committee (CMO Regio Arnhem-Nijmegen) approved the study protocol and amendments. Written informed consent was obtained from all patients. The study was registered at ClinicalTrials.gov (NCT00860860; http://www.clinicaltrials.gov/ct2/results?term=NCT00860860).

Study design. Preclinical studies showed successful pretargeting of tumours depends on three factors: the bsMAb dose, the interval between the administration of the bsMAb and the radiolabelled hapten-peptide, and the dose of the hapten peptide (Sharkey *et al*, 2003a; Schoffelen *et al*, 2010b). Tumour targeting of the radiolabelled hapten peptide will be affected by: (1) the amount of bsMAb in the tumour, which should be high enough to provide efficient capture of the radiolabelled peptide and (2) the bsMAb concentration in the circulation, which should be low at the time of the radiolabelled hapten peptide administration to prevent extensive complex formation with the bsMAb. Complex formation would increase the circulatory half-life of the radiolabelled hapten peptide and in turn increasing the radiation exposure to normal tissues. Additionally, animal studies showed that the dose of the hapten peptide should be minimised to increase the fraction that targets the tumour (Schoffelen *et al*, 2010b). For this clinical trial, the IMP288 dose was selected based on the minimum amount required to radiolabel the peptide with 7.4 GBq ^{177}Lu (maximum specific activity 74 MBq μg^{-1}, therefore 100 μg (68.6 nmol) was required). Animal data also had indicated the molar ratio between the TF2 and IMP288 dose should be at least 10 : 1, and therefore our goal was to adjust the bsMAb and peptide to achieve this minimum ratio (Schoffelen *et al*, 2010b).

To evaluate these interdependent factors relevant for tumour targeting, we studied four dose schedules in cohorts of five patients (Table 1). First, the effect of the interval between bsMAb and hapten peptide was studied: cohort 1 received TF2 (75 mg; 477 nmol) and IMP288 (100 μg; 68.6 nmol) with a 5-day interval, while cohort 2 received the same doses with a 1-day interval. The 5-day interval was based on preclinical studies that showed a substantially slower blood clearance rate for TF2 in rabbits than in mice, and thus for the first-in-man experience, the longer interval was chosen for the first cohort (Sharkey *et al*, 2010). Once the rapid clearance rate of TF2 was determined, it was apparent that a 1-day spacing would be suitable, and thus all other cohorts used this interval. The bsMAb dose also was examined, with cohort 3 receiving a higher bsMAb dose (150 mg TF2, 1-day interval, 100 μg IMP288), while all other cohorts received 75 mg of TF2. Finally, with the availability of ^{177}Lu at a higher specific activity, in cohort 4, it was possible to test a lower IMP288 dose (75 mg TF2, 25 μg IMP288, 1-day interval), with 25 μg being the minimum amount of IMP288 required to prepare the maximum amount of ^{177}Lu activity (7.4 GBq) per treatment.

Patients first underwent a diagnostic imaging cycle with TF2 and ^{111}In-labelled IMP288. If tumour targeting of the radiolabelled peptide was observed, then patients received a therapeutic cycle of TF2 and ^{177}Lu-IMP288 the following week (Figure 1). By quantitatively analysing the ^{111}In-IMP288 scintigraphic images and pharmacokinetics, the radiation dose to the kidney and bone marrow was estimated. The amount of ^{177}Lu-IMP288 activity to be given as the therapeutic dose was set on an individual patient basis, designed to deliver no $>$1.25 Gy to the bone marrow or 15 Gy to the kidneys. This total dose was split into four equally divided treatment cycles, with the intent to administer each cycle every 8 weeks, since the nadir for directly radiolabelled IgG therapy usually occurs within 5–6 weeks. The maximum allowed dose per cycle was set initially for cohort 1 at 3.7 GBq, but was increased to 7.4 GBq for all subsequent cycles.

Preparation and administration of investigational drugs. The clinical-grade bsMAb, TF2, and the IMP288 hapten peptide were

Table 1. Study design

Cohort (n = 5)	TF2 (mg)	Interval (days)	IMP288 (μg)
1	75	5	100
2	75	1	100
3	150	1	100
4	75	1	25

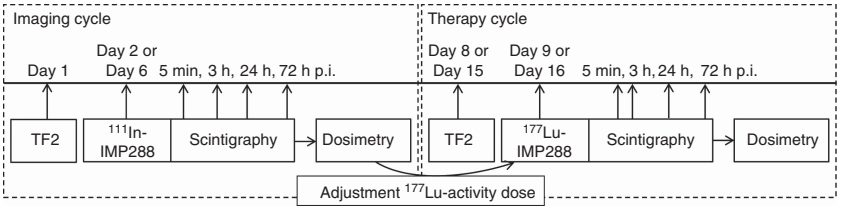

Figure 1. Treatment schedule. Patients received an imaging cycle with TF2 and ^{111}In-IMP288 to determine the pharmacokinetics and radiation dose to the red bone marrow and kidneys. A safe, cumulative ^{177}Lu-activity dose was estimated, and one-fourth of this amount was administered in the first therapy cycle.

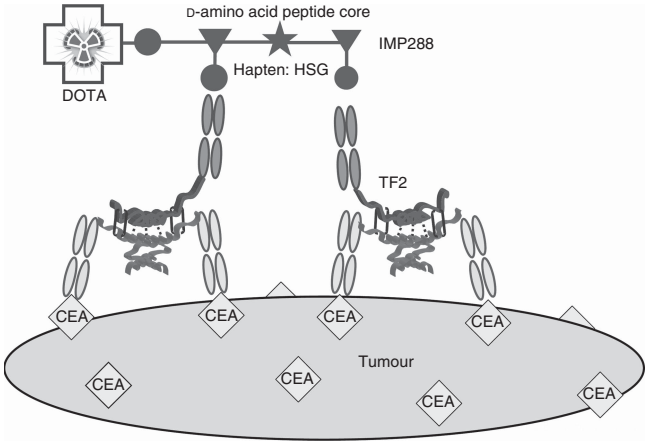

Figure 2. Schematic representation of the pretargeting agents. The trivalent bispecific antibody construct, TF2, binds divalently to CEACAM5, the tumour-associated antigen that is overexpressed on the cell surface of colorectal tumour cells. After the bsMAb has localised the tumour and cleared from the blood, a radiolabelled divalent peptide is given, substituted with the hapten, histamine-succinyl-glycine (HSG). This is rapidly targeted to the tumours and bound by high affinity to the anti-HSG Fab fragment of the bsMAb. Due to its bivalency, it has the ability to crosslink the bsMAbs at the tumour surface, forming a stable complex. The peptide is conjugated with the chelator, DOTA that can be labelled with a variety of radionuclides.

provided by Immunomedics (Morris Plains, NJ, USA) (Figure 2). The binding characteristics of the bispecific antibody, TF2, were described previously (Rossi *et al*, 2006). IMP288 (molecular weight 1456 Da) was synthesised as described by McBride *et al* (2006). TF2 was diluted in 60 ml 0.9% w/v NaCl, and administered by i.v. infusion over a period of 2 h. Starting from the second patient of cohort 2, all patients received a prophylactic dose of the anti-histamine, clemastine (2 mg) i.v. 15 min before start of the second TF2 infusion. Dexamethasone (10 mg i.v.) was added subsequently as an additional prophylactic medication before the second TF2 infusion, starting from the last patient of cohort 3.

IMP288 was labelled as described previously (Schoffelen *et al*, 2010b), either with 185 MBq ^{111}In, a gamma-emitter for pre-therapy imaging study or with 3.7–7.4 GBq ^{177}Lu, a therapeutic beta-emitter that also has a gamma emission capable of being imaged. The radiochemical purity was $>95\%$ for all preparations. ^{111}In- and ^{177}Lu-IMP288 were diluted in 10 or 20 ml of 0.9% NaCl, respectively, and were administered by an intravenous 2-min bolus injection.

Scintigraphic assessment and analysis. Anterior and posterior whole-body planar scintigraphic images (Siemens Ecam, Hoffmann Estates, IL, USA) were acquired at 5 min, 3 h, 24 h, and 72 h after injection of IMP288. SPECT scans were also taken of regions where $\geqslant 1$ tumours were in the field of view.

Radioactivity concentrations were determined by drawing regions of interest at tumours and muscle in the psoas region in the SPECTs that were acquired 24 h after injection of ^{111}In-IMP288. Tumour-to-normal tissue ratios were calculated.

Patient evaluation and follow-up. Toxicity assessment, haematology, clinical biochemistry, physical examination, and ECOG performance status were performed at baseline and weekly during follow-up, up to 8 weeks after therapy. Urinary analysis was performed weekly to monitor proteinuria. Patients were monitored closely during and up to 5 h after the TF2 infusions and IMP288 injections. Toxicity was evaluated according to the NCI Common Terminology Criteria for Adverse Events v3.0 (NCI-CTCAE).

To evaluate tumour response, a baseline FDG-PET/CT (contrast-enhanced) scan was performed within 2 weeks before therapy, and 8 weeks after the ^{177}Lu-IMP288 injection. Responses were evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST) (Eisenhauer *et al*, 2009).

Pharmacokinetics. TF2 concentrations in serum samples were determined using a sandwich enzyme-linked immunosorbent assay (ELISA), using plates coated with an HSG-conjugated peptide coupled to bovine serum albumin. After incubating with dilutions of the patient's serum samples, binding was revealed with an anti-idiotype antibody directed against the humanised anti-CEACAM5 portion of TF2 (Sharkey *et al*, 2010).

^{111}In- and ^{177}Lu-IMP288 pharmacokinetics were determined by measuring the radioactivity in blood samples in a gamma counter along with standards prepared from the injected products for determination of the percentage of the injected dose per gram tissue (% ID g^{-1}).

Human-anti-human antibody measurements. Human-anti-human antibody (HAHA) was determined before each TF2 infusion and up to 8 weeks after the last TF2 infusion. The HAHA directed against TF2 was measured with a sandwich ELISA. Serial dilution of patient serum was incubated in TF2-coated wells. Wells were probed with a TF2-horseradish peroxidase conjugate, and binding was revealed using o-phenylenediamine dihydrochlorides. Concentration of anti-TF2 responses (ng ml^{-1}) was based on a standard curve using a rat anti-idiotype antibody specific to hMN-14, WI2 (Losman *et al*, 1994). The detection limit of the assay is 50 ng ml^{-1}, and therefore a positive HAHA was arbitrarily set as any value above this level.

RESULTS

Twenty-one patients were enrolled in the study between July 2009 and July 2011. Baseline characteristics of the patients who received TF2 ^{111}In/^{177}Lu-IMP288 cycles are reported in Table 2. One patient (patient 1) was withdrawn from the study before the ^{177}Lu-IMP288 treatment. He experienced a hypoxia grade 2 during the second TF2 infusion; and therefore, the infusion was discontinued and the patient was withdrawn.

Table 2. Patient characteristics	
Age, median (range)	63 (39–76) years
Male/female	12/9
Performance score	
0	8 (38%)
1	13 (62%)
Site of primary tumour	
Colon	15 (71%)
Rectum	6 (29%)
Site of disease	
Primary	8 (38%)
Liver	18 (86%)
Lungs	14 (67%)
Lymph nodes	8 (38%)
Bones	4 (19%)
Soft tissue	3 (14%)
Peritoneum	1 (5%)
Tumour load	
Baseline sum diameters of all lesions per patient, median (range)	27.6 (9.2–111.0) cm
Prior treatment	
Surgery	17 (81%)
Chemotherapy	21 (100%)
Bevacizumab	17 (81%)
Anti-EGFR therapy	7 (33%)
External radiotherapy	6 (29%)
CEA plasma level	
Baseline, median (range)	120 (12–2200) $\mu g\,l^{-1}$
Administered ^{177}Lu-activity dose	
Median (range)	5.6 (2.5–7.4) GBq

Abbreviations: CEA=carcinoembryonic antigen; EGFR=epidermal growth factor receptor.

Most patients had large tumour loads, with many large lesions in multiple organs. The low estimated red marrow doses allowed administration of the maximum ^{177}Lu-IMP288 dose in a number of patients (e.g., 4, 2, and 3 patients in cohort 2 through 4, respectively, received 7.4 GBq). The other patients in cohorts 2–4 received ^{177}Lu-activity doses ranging from 2.5 to 6.2 GBq to avoid exceeding one-fourth of the maximum cumulative red marrow absorbed dose (1.25 Gy). The renal dose threshold was never an issue for assignment of the therapeutic dose.

Pharmacokinetics. Measurements of ELISA indicated that TF2 cleared rapidly from the blood, with 86% of the ID eliminated at 6 h after completion of the infusion, and 99% ID after 24 h in all cohorts. TF2 clearance was not related to the CEA plasma level at the time of infusion (data not shown). TF2 concentrations in the blood increased proportionally with the dose administered (Figure 3a).

Radiolabelled IMP288 cleared the fastest in cohort 1, where the initial interval was 5 days (Figure 3b). Because TF2 cleared quickly, the interval was then adjusted to 1 day for cohort 2. In this cohort, the clearance rate of IMP288 slowed, but it was still very rapid. As expected, IMP288 clearance slowed when conditions favoured a higher ratio of moles of TF2 in the blood to the moles of IMP288 injected. For example, IMP288 concentrations were higher in cohort 3 that received 150 mg than in patients of cohort 2 that received 75 mg (Figure 3b). Overall, most of the administered radioactivity had cleared from the blood at 24 h post injection in all cohorts (100%, 99%, 98%, and 98% ID, cohorts 1–4, respectively).

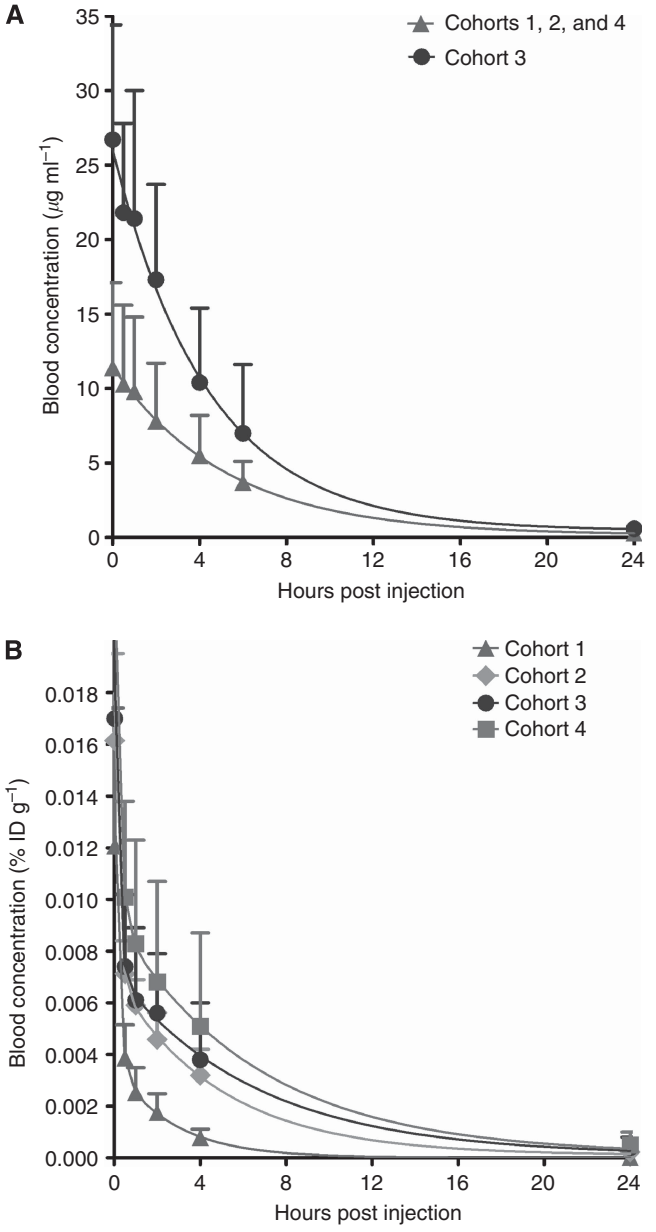

Figure 3. Pharmacokinetics. (**A**) Serum clearance of TF2 determined by ELISA in cohorts 1, 2, and 4 that received 75 mg of TF2, and in cohort 3, 150 mg (mean ± standard deviation; $N=5$ per cohort). TF2 cleared rapidly from the serum, with cohort 3 having twice as high serum concentrations. (**B**) ^{111}In-IMP288 blood clearance per cohort (mean ± standard deviation; $N=5$ per cohort). In all cohorts, >98% ID was cleared at 24 h p.i., although peptide blood clearance was somewhat delayed by shortening the interval between bsMAb and peptide administration, and to a lesser extent due to a higher antibody and a lower peptide doses.

Scintigraphic imaging analysis. In all patients, the ^{111}In-IMP288 images showed clear and selective targeting of known tumour lesions; and thus, all patients were eligible to receive a therapeutic TF2/^{177}Lu-IMP288 cycle. Primary tumours, as well as metastases in the lungs, liver, lymph nodes, and soft tissue, were visualised as early as 1 h after injection. Representative images of patient 21 in cohort 4 are shown in Figure 4.

The pretherapy ^{111}In scans and the posttherapy ^{177}Lu scans were congruent, with a somewhat stronger signal in the ^{177}Lu scans due to higher levels of activity given. After 1 day, most activity had cleared from the normal tissues, with very limited retention in the kidneys, resulting in high tumour-to-normal tissue ratios ($>20:1$ at 24 h) in all cohorts (Table 3). Shortening the interval between the bsMAb and peptide administration in cohort 2 resulted in significantly higher absolute activity concentrations in all tumours compared with cohort 1 ($P=0.0079$) and in higher tumour-to-normal tissue ratios ($P=0.046$). Furthermore, all patients in cohort 4 with liver metastases ($n=3$) appeared to have higher tumour activity concentrations (6.3, 9.7, and 23.6% ID per kg) and tumour-to-normal tissue ratios (33, 38, and 84) than the patients in cohort 2 with liver metastases ($n=4$) (tumour activity concentrations: 3.2, 4.7, 5.4, and 5.7% ID per kg, and tumour-to-normal tissue ratios: 20, 24, 27, and 29 at 24 h p.i.), suggesting improved tumour targeting at the lower peptide dose (25 *vs* 100 μg).

Overall, the combined effect of shortening the interval and reducing the peptide dose resulted in significantly higher tumour activity concentrations and tumour-to-normal tissue ratios (cohort 1 *vs* cohort 4, $P=0.0079$ and $P=0.035$, respectively).

Efficacy, safety, and tolerability. According to RECIST based on the FDG-PET/contrast-enhanced CT scans before therapy and 8 weeks after therapy, all patients showed progressive disease 8 weeks after the first therapy cycle with TF2 and ^{177}Lu-IMP288; and therefore, none of the patients were eligible for a second treatment cycle. Thus, only the safety and tolerability of one of the four planned treatment cycles can be reported.

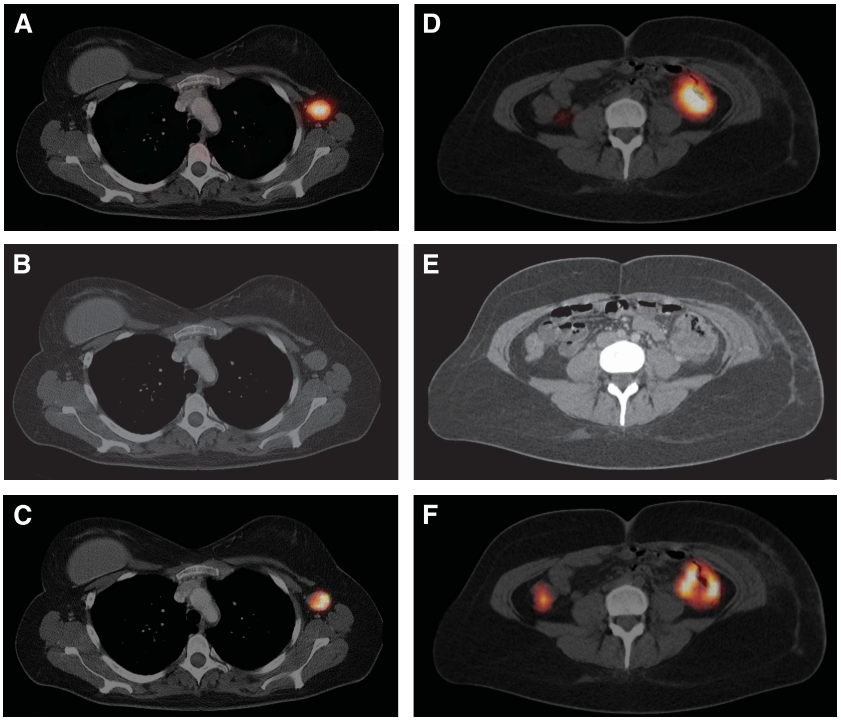

Figure 4. Scintigraphic images. The SPECT/CT image (**A**), acquired 24 h after injection of ^{111}In-IMP288 (185 MBq, 25 μg), pretargeted with 75 mg TF2 (1-day interval), in a 38-year-old patient (cohort 4), shows very clear tumour targeting of an axillary lymph-node metastasis, with very low concentrations of radioactivity in normal tissues. This patient had a CEA level of 17 μg l^{-1} and the concentration of ^{111}In-IMP288 in the tumour was 6.3% ID kg^{-1}, with a tumour-to-normal tissue ratio of 33. Corresponding contrast-enhanced CT scan and a fused FDG-PET/CT scan are shown (**B** and **C**, respectively). The primary colon tumour (50 cm ab ano) also shows highly specific tumour targeting in the SPECT image (**D**), confirmed by the CT scan and FDG-PET/CT (**E** and **F**, respectively), with non-specific FDG uptake in the ascending colon.

Table 3. Tumour targeting

	bsMAb dose/interval/peptide dose			
	75 mg/5 days/100 μg Cohort 1	**75 mg/1 day/100 μg Cohort 2**	**150 mg/1 day/100 μg Cohort 3**	**75 mg/1 day/25 μg Cohort 4**
Activity concentration (%ID kg^{-1}, mean (range))				
Tumour	1.4 (0.8–2.0)	4.4 (2.7–5.7)	5.9 (3.1–10.4)	9.4 (2.2–23.6)
Normal tissue	0.068 (0.042–0.093)	0.18 (0.12–0.24)	0.19 (0.08–0.31)	0.21 (0.10–0.28)
Tumour-to-normal tissue ratio (mean (range))	20 (17–22)	24 (20–29)	32 (22–43)	40 (21–84)

Abbreviation: ID = injected dose.

Toxicity was limited in most patients, with no apparent differences between cohorts (Table 4). The majority of the patients with liver metastases had liver enzyme elevations before drug administrations (e.g., 43% had grades 3–4 GGT elevation at baseline). In many patients, GGT increased during the trial, which was deemed to be disease related, since all patients had progression of liver metastases, as seen on the FDG-PET/CT scans.

Seven patients (33%) experienced a mild grade 2 infusion reaction during onset of their second TF2 infusion. They experienced flushes, dyspnoea, chest pain, back pain, or coughing. They did not have cutaneous, cardiovascular, or gastrointestinal signs or symptoms. All reactions were easily controlled by interrupting the infusion, and with intravenous administration of clemastine (2 mg) and dexamethasone (10 mg). Except for patient 1, with grade 2 hypoxia, the infusion was restarted in all patients at a slower infusion rate for 15 min, and could be completed at the planned infusion rate without any recurrence of symptoms. After three infusion reactions (from patient 8), prophylactic intravenous clemastine (2 mg) was added before the patients' second TF2 infusion. After patient 15, prophylactic intravenous dexamethasone (10 mg) was added. With this regimen, infusion reactions occurred in 2 out of 5 patients, but the TF2 infusions could be completed as described above.

Following the single therapy cycle, bone marrow toxicity was mild in most patients (grades 1–2 in 30% of patients). More severe haematological toxicity (grades 3–4 thrombocytopaenia, and grade 3 lymphopaenia) occurred in two patients (10%; 1 in cohort 2 and 1 in cohort 3), with the nadir 5–6 weeks after ^{177}Lu administration. Recovery was rapid, returning to grade $\leqslant 1$ level at 7–8 weeks after therapy. None of these patients had complications or needed intervention. None of the patients showed signs or symptoms of renal toxicity.

One patient (in cohort 3) was admitted to the hospital due to severe dyspnoea 4 days after the administration of ^{177}Lu-IMP288. High-resolution CT of the chest was unremarkable, lung function tests were normal and blood or sputum cultures, as well as viral serology, remained negative. The event was reported as probably related to the drug administrations, either TF2 or ^{177}Lu-IMP288, and thus recorded as a Suspected Unexpected Serious Adverse Reaction (SUSAR). The patient's dyspnoea decreased, but remained at a lower level, which might have been related to rapid progression of disease, since this patient had progressive lung metastases.

Human antibodies against TF2 > 50 ng ml^{-1} were detected in 11 of 21 patients, starting 1 week after the second TF2 infusion, and gradually increasing in the follow-up period of 8 weeks, indicating that the humanised trivalent bsMAb TF2 was immunogenic in $\sim$50% of the patients upon repeated injection. Titers varied widely among patients (mean 386 ng ml^{-1}, range 53–800 ng ml^{-1}).

Table 4. Adverse events

		Grade			
		2	3	4	Total of grades 2–4
Category	**Adverse event**	**(No. of patients)**			**(%)**
Blood/bone marrow	Platelets	1	1	1	14
	Lymphocytopaenia	1	2		14
	Haemoglobin	2	1		19
Syndromes	Acute infusion-related reaction	7			33
Constitutional symptoms	Fatigue	4			19
	Fever	1			5
	Sweating	1			5
Pulmonary	Dyspnoea	1	1		10
Gastrointestinal	Nausea	1			5
	Anorexia	4			19
	Diarrhea		2		10
Pain	Abdomen	3	1		19
	Tumour	1			5
Neurology	Somnolence	2			10
	Pyramidal tract dysfunction[a]	1			5
Infection	Biliary tree[b]		1		5
Musculoskeletal	Arthritis (non-septic)	1			5
Renal	Urinary frequency	1			5
Metabolic/laboratory	AST	4	1		24
	ALT	2	1		14
	Bilirubin	1	2		14
	Albumin, serum low	6			29
	Alkaline phosphatase	5	5		48
	GGT	4	7	3	67

Abbreviations: ALT = alanine transaminase; AST = aspartate transaminase; CT = computed tomography; GGT = gamma-glutamyl transpeptidase; MRI = magnetic resonance imaging; PET = positron emission tomography.

[a]Brain metastases confirmed by MRI cerebrum.

[b]Liver metastases with biliary tract obstruction confirmed by PET/CT.

DISCUSSION

Pretargeting aims to improve the efficacy of tumour targeting with monoclonal antibodies. Recently, a survival benefit for patients with medullary thyroid carcinoma following treatment with pretargeted RIT with an ^{131}I-di-DTPA peptide (1.9–5.5 GBq) was reported (Chatal *et al*, 2006).

Preclinical studies with the pretargeting system used in this study (Rossi *et al*, 2006; Goldenberg *et al*, 2008) have shown that this system can used for PET or SPECT imaging (McBride *et al*, 2006; Schoffelen *et al*, 2010a), and for pretargeted RIT (Sharkey *et al*, 2003b; Schoffelen *et al*, 2010b). These studies showed that accurate dosing of the bsMAb and the radiolabelled peptide is crucial to obtain optimal tumour targeting (Schoffelen *et al*, 2010b).

In this first-in-man pretargeting study with TF2 and ^{111}In/^{177}Lu-labelled IMP288, we showed that pretargeting provided rapid and efficient targeting of CEACAM5-expressing tumours with low normal tissue activity levels. The main objective of this study was to evaluate several dosing conditions to assess their effect on biodistribution and tumour targeting. The interval of 5 days that was applied in the first cohort was selected based on blood clearance studies of TF2 in rabbits (Sharkey *et al*, 2010), but when it became clear that TF2 cleared relatively rapidly in humans, the interval was reduced to 1 day. The blood clearance of TF2 was much faster than that of similarly sized IgG molecules, which could be explained by the fact that TF2 lacks a C_{H2} domain (Chinn *et al*, 2006). In nude mouse-human tumour xenograft models, an interval of 16–24 h was optimal (Sharkey *et al*, 2003a; Schoffelen *et al*, 2010b). In addition, TF2's peak tumour uptake occurred $\sim$6 h post injection in these models, decreasing gradually over time, and thus minimising the interval allowed the hapten peptide to be captured when more bsMAb is present in the tumour.

Overall, we found that reducing the interval and the IMP288 dose improved tumour targeting. Higher bsMAb doses also can enhance tumour uptake of the radiolabelled peptide (Schoffelen *et al*, 2010a, b). We did not observe improved tumour uptake by increasing the TF2 dose four-fold (75–150 mg). We did observe a trend towards increased tumour uptake when the IMP288 dose was lowered from 100 to 25 μg using 75 mg of TF2 (e.g., cohort 2 *vs* cohort 4). Further modifications might yield additional improvements in tumour uptake while minimising normal tissue uptake; however, such improvements also must be balanced against toxicity. In this study, haematological toxicity appeared to be the most likely dose-limiting effect, since renal doses remained low for all dose levels.

This study demonstrated the safety of pretargeted RIT with TF2 at activity doses of ^{177}Lu-IMP288 ranging from 2.5 to 7.4 GBq. The immune responses, that is, symptomatic infusion reactions and the formation of anti-TF2 antibodies that were observed following the administration of the second TF2 infusion were unexpected, since TF2 is a humanised antibody construct that also lack an Fc moiety. Murine precursors of anti-CEA bsMAb frequently showed immune responses (Kraeber-Bodere *et al*, 1999; Vuillez *et al*, 1999), which was reduced by using chimaeric and humanised antibodies. Importantly, the mild, grade 2, acute infusion-related reactions that were observed in one-third of the patients at the second infusion of the humanised bsMAb did not preclude continuation of treatment, except for one patient who had extensive pulmonary metastases. We observed that reducing the infusion rate and the preadministration of prophylactic antihistamines and corticosteroids reduced this adverse event, and this is advised for future studies. The human antibodies against TF2 detected in half of the patients were not present at the time of the second TF2 infusion (i.e., therapy cycle), so TF2's clearance was not affected. No correlation was found between the infusion reactions and the anti-TF2 antibody titers that started to increase within 1 week after the second TF2 infusion. Future studies should consider a more condensed treatment regimen to minimise any impact that HAHA might have on safety and the quality of tumour targeting.

While haematological toxicity of pretargeted RIT was the more apparent event related to the ^{177}Lu-IMP288 exposure, overall it was minimal, particularly when considering that these patients all had received several lines of chemotherapy and up to 7.4 GBq of ^{177}Lu-IMP288. Indeed, although the two patients with transient grade $\geqslant$3 bone marrow toxicity had a somewhat higher bone marrow absorbed dose, the radiation dose to the red marrow was very low, and therefore we suspect that underlying patient-specific factors (age, performance status, effects of prior treatments on haematopoietic stem cell reserve) likely contributed to these toxicities. The dosimetric analysis has been reported previously (Schoffelen *et al*, 2011), and will be described in more detail elsewhere.

This trial was designed with the intent to administer high levels of ^{177}Lu-IMP288 using dosimetry to predict a safe dose. The radiolabelled hapten peptide used in pretargeting can be viewed in a similar manner as radiolabelled peptides that are being used to treat neuroendocrine tumours (Baum and Rosch, 2011), where dosimetry has gained a role in predicting the potential for renal toxicity. In our study, we determined the total therapeutic dose based on a pretherapy imaging study using conservative estimates of the red marrow and renal doses that should not be exceeded. However, for additional safety, this total dose was split into four fractions, allowing sufficient time between each treatment to monitor toxicity, primarily haematological toxicity. Unfortunately our study population had extensive metastatic disease, and thus all patients showed disease progression before additional treatment cycles could be given. This trial was also designed for use with ^{177}Lu-IMP288 with an eye to the future application of pretargeted RIT to patients with less bulky disease, since RIT has been shown to be more effective in small-volume disease (Jain, 1990; Liersch *et al*, 2005). Recent clinical data in patients with advanced pancreatic cancer suggest a fractionated dosing regimen using a ^{90}Y-labelled antibody given in combination with low-dose (radiosensitising) gemcitabine can provide disease control and even objective responses (Ocean *et al*, 2012), giving credibility to pursuing ^{90}Y instead of ^{177}Lu for patients with advanced metastatic disease. Indeed, ^{90}Y's physical half-life (64 h) matches the residence time in the tumour better than ^{177}Lu-IMP288 (6.7 days).

In conclusion, the results of this phase I clinical study with pretargeted RIT showed rapid and specific tumour targeting of the ^{111}In- or ^{177}Lu-hapten peptide IMP288 following pretargeting with the bsMAb TF2. Tumour targeting improved by shortening the interval between the bsMAb and peptide administration, and by lowering the peptide dose. The procedure is safe, and infusion reactions are transient and manageable with appropriate medication and lowering the infusion rate. Further studies will be needed to determine whether improvements in targeting can be obtained by additional adjustments to the pretargeting conditions, as well as revising the protocol design to allow full treatment to be given over a shorter duration.

ACKNOWLEDGEMENTS

This study was supported by Dutch Cancer Society grant no KUN 2008-4038 and NIH grant 1 R43 EB003751.

CONFLICT OF INTEREST

WJ McBride, DM Goldenberg, and EA Rossi are employed by or have a financial interest in Immunomedics, Inc.

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