

Original Article

Phase I trial to evaluate the tumor and normal tissue uptake, radiation dosimetry and safety of ^{111}In -DTPA-human epidermal growth factor in patients with metastatic EGFR-positive breast cancer

Katherine A Vallis¹, Raymond M Reilly^{2,3,4}, Deborah Scollard², Pat Merante⁵, Anthony Brade⁵, Sobi Velauthapillai², Curtis Caldwell^{4,6}, Ida Chan⁴, Marc Freeman⁴, Gina Lockwood⁷, Naomi A Miller⁸, Bart Cornelissen¹, Jennifer Petronis⁹, Kathryn Sabate⁷

¹Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, UK; ²Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada; ³Toronto General Hospital Research Institute, Toronto, Canada; ⁴Medical Imaging, University of Toronto, Toronto, Canada; ⁵Radiation Medicine Program, Princess Margaret Hospital, University of Toronto, Canada; ⁶Medical Physics, Sunnybrook Health Sciences Center, Toronto, Canada; ⁷Biostatistics, Princess Margaret Hospital, Toronto, Canada; ⁸Pathology, University Health Network, Toronto, Canada; ⁹Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, Canada

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Abstract: The safety, pharmacokinetics, biodistribution and radiation dosimetry of ^{111}In -DTPA-hEGF, an Auger electron-emitting radiopharmaceutical, were evaluated in a first-in-human trial. Dose escalation was performed in patients with EGFR-positive metastatic breast cancer who had received ≥ 2 prior courses of systemic treatment. ^{111}In -DTPA-hEGF (0.25 mg) was administered once intravenously (i.v.). Blood was collected for biochemistry/hematology testing and pharmacokinetic and immunogenicity analyses at selected times post injection (p.i.). Whole body planar images were acquired at 1, 4-6, 24 and 72 h p.i. and SPECT images at 24 and/or 72 h p.i. Macrodosimetry (MIRD) for the whole body and organs was estimated using OLINDA. Correlative radiological imaging was obtained at baseline, 1 and 3 months and then 6 monthly. Toxicity was scored using Common Terminology Criteria for Adverse Events (CTCAE)v2.0. Sixteen patients, median age 47 yr (range, 35-59), received ^{111}In -DTPA-hEGF as follows: 357-434 MBq (7), 754-805 MBq (3), 1,241-1,527 MBq (3) and 2,030-2,290 MBq (3). Fifteen were evaluable for toxicity. The commonest adverse events (AE) were flushing, chills, nausea, and vomiting occurring during or immediately p.i. One patient experienced Grade 3 thrombocytopenia (attributed to bone marrow infiltration by cancer). There were no other Grade 3 or 4 AEs. Maximum tolerated dose was not reached. Clear accumulation of radiopharmaceutical in at least one known site of disease was observed in 47% of patients. ^{111}In -DTPA-hEGF was cleared biexponentially from the blood with α -phase $T_{1/2}$ of 0.16 ± 0.03 h and β -phase $T_{1/2}$ of 9.41 ± 1.93 h. ^{111}In -DTPA-hEGF was not immunogenic. The mean radiation dose estimates in mGy/MBq for whole body, liver, kidneys, spleen and thyroid were 0.08, 0.86, 0.74, 0.37 and 0.30, respectively. No objective antitumor responses were observed at the doses studied. In summary, administered amounts of up to 2,290 MBq (0.25 mg) of ^{111}In -DTPA-hEGF were well tolerated as a single i.v. injection.

Keywords: Auger electron, ^{111}In -DTPA-hEGF, breast cancer, Phase I trial

Introduction

The epidermal growth factor receptor (EGFR) is a cell surface signaling glycoprotein that contains extracellular EGF-binding, transmembrane and cytoplasmic domains. It has tyrosine kinase activity and undergoes autophosphorylation following ligand binding. Upregulation of EGFR has been demonstrated in many human

cancers including breast carcinoma [1]. Since overexpression of EGFR plays a role in proliferation and is associated with poor prognosis, it is an attractive therapeutic target [2]. Interventions exploiting overexpression of EGFR include antibodies that block ligand-binding and tyrosine kinase inhibitors that interfere with receptor autophosphorylation and mitogenic signaling [3]. These EGFR-targeted agents

are of limited efficacy in breast cancer although their role in triple negative breast cancer, in which EGFR is frequently overexpressed, is being evaluated [1]. We investigated a strategy for EGFR-targeting which, unlike small molecule and antibody inhibitors, does not rely on inhibition of receptor activation, but uses EGF conjugated to ^{111}In (^{111}In -DTPA-hEGF) to exploit the EGFR as a means of transporting radioactivity into cancer cells [4]. ^{111}In emits nanometer-range Auger and micrometer-range conversion electrons, which are densely ionizing, making it an attractive alternative to beta-emitting isotopes for targeted radiotherapy. On binding the EGFR, ^{111}In -DTPA-hEGF is internalized and delivered to the cell nucleus where it leads to DNA damage and cell death. Normal cells, with modest or absent EGFR expression, do not internalize or internalize less ^{111}In -DTPA-hEGF and are therefore relatively resistant to its cytotoxic effect [5]. The use of EGF labeled with ^{131}I to target tumors was reported previously [6]. However, efficient internalization of radioiodinated peptides results in loss of radiolabel from tumor cells due to deiodination. In contrast, intracellular retention of ^{111}In is prolonged, a significant advantage of this radionuclide for imaging and Auger electron radiotherapy [7].

^{111}In -DTPA-hEGF was cytotoxic in MDA-MB-468 human breast cancer cells that overexpress EGFR in vitro and inhibited the growth of MDA-MB-468 tumor xenografts implanted subcutaneously (s.c.) into athymic mice [5, 8]. Mice received a total of 27.7, 55.5 or 92.5 MBq ^{111}In -DTPA-hEGF split over 5 weekly doses. Significant anti-tumor effects were observed even in the lowest dose group at 7 weeks after treatment [8].

To assess the toxicity of ^{111}In -DTPA-hEGF, female BALB/c mice received ^{111}In -DTPA-hEGF (44.4 MBq; equivalent to 8.5 GBq in humans) by intravenous (i.v.) injection [8]. After 15 days no significant changes, relative to controls, were observed in hematological parameters (RBC, WBC, platelet counts and hemoglobin), biochemical parameters (plasma alanine transaminase [ALT] and creatinine [Cr]) or in body weight. Histopathological assessment of 19 tissues revealed no significant abnormalities. To assess the toxicity of repeat dosing athymic mice bearing MDA-MB-468 xenografts received five injections of ^{111}In -DTPA-hEGF (18.5 MBq) at weekly intervals. No significant differences in

ALT or Cr and no pathological changes in the liver, kidneys or heart were observed 7 weeks after treatment. There was a small decrease in WBC and platelet counts in treated mice but these remained within the normal range.

^{111}In -DTPA-hEGF was formulated for human administration [9]. Patients with EGFR-positive metastatic breast cancer received ^{111}In -DTPA-hEGF i.v. on a single occasion. The administered amount was escalated in groups of 3-7 patients. The aims of the trial were to investigate the pharmacokinetics, toxicity profile and biodistribution of ^{111}In -DTPA-hEGF in this first-in-human study.

Materials and methods

Study participants and design

Study participants attending the Princess Margaret Hospital and Odette Cancer Centre, Toronto, were recruited after giving written informed consent. Separate consent was obtained for pre-screening of archived tumor samples for EGFR expression by immunohistochemistry. Study participants aged 18-80 years with Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 were eligible. Histologic diagnosis of invasive breast carcinoma with positive immunostaining for EGFR and radiologically assessable disease were required. Serum bilirubin, Cr, leukocyte and platelet counts and hemoglobin were required to be normal. Alkaline phosphatase (ALP) and hepatic transaminases were required to be less than twice the upper limit of normal. Study participants had received at least two chemotherapy/hormonal regimens for metastatic disease and were excluded if they had received chemotherapy up to four weeks previously. This study was approved by the Research Ethics Board at University Health Network (UHN), Toronto (reference: 02-0788-C) and received Clinical Trial Application (CTA) approval by Health Canada (CTA number 085648).

An up-and-down dose-finding design, with study participants treated in cohorts of at least three, was used. Each participant received a single administration of ^{111}In -DTPA-hEGF. The radioactivity levels administered were 370, 740, 1,480 and 2,220 MBq (6.2-37 MBq/kg, assuming an average female weight of 60 kg). The starting level of ^{111}In (370 MBq) was select-

ed as it is close to the maximum dose of ^{111}In -pentetreotide used for imaging neuroendocrine tumors (300 MBq). This amount of ^{111}In was therefore known to be safe. The highest amount of radioactivity (2,220 MBq, 37.0 MBq/kg) corresponded to the equivalent maximum dose of radioactivity safely administered by i.v. injection to female white New Zealand rabbits (i.e. 42.5 MBq/kg) in pre-clinical toxicology studies [4]. The mass amount of hEGF that was administered was fixed at 0.25 mg per administration. These dose limits were restricted by the Health Canada approval of the CTA since ^{111}In -DTPA-hEGF had not previously been administered to humans.

Vital signs were recorded at baseline and at 5, 15 and 30 min and 1, 2, 4-6, 24, 48 and 72 h post-injection (p.i.). Hematological and biochemical analyses were performed at baseline, at 1 week and 1, 2, 3 and 6 months and then 6 monthly p.i. Adverse events (AE) were recorded at 10 min, 1 h, 7 days and 1, 2 and 3 months p.i. and every 3-6 months thereafter. Toxicity was graded using the Common Toxicity Criteria for AEs version 2.0 (CTCAEv2.0; National Cancer Institute, Bethesda, MD) [10]. Dose limiting toxicity (DLT) was defined as Grade 3 or 4 hematologic or non-hematologic toxicity. Blood was collected at baseline, 5, 15, 30 minutes and 1, 2, 4-6, 24, 48 and 72 h p.i. for pharmacokinetic analysis. Whole-body planar images were acquired at 1, 4-6, 24 and 72 h p.i. and SPECT images at 24 h and in some cases at 72 h. Macrodosimetry (Medical Internal Radiation Dose [MIRD]) estimates were calculated for the whole body and selected organs using the Organ Level Internal Dose Assessment/Exponential Modeling code (OLINDA/EXM) [11]. CT scans were performed within 4 weeks before treatment and at 4 and 12 weeks p.i. and 6 monthly thereafter for Response Evaluation Criteria In Solid Tumors (RECIST) assessment [12].

Tumor EGFR expression

Archived primary tumors of potential study subjects were screened for EGFR-positivity. Histologic sections (5 μm) were prepared from formalin-fixed, paraffin-embedded tumor blocks. Immunohistochemical staining was performed using anti-EGFR monoclonal antibody (Clone 31G7, Zymed Laboratories) at 1:100 dilution. All slides were reviewed by a patholo-

gist (N.M). A tumor was considered EGFR-positive if staining of any intensity of cytoplasm or cell membrane was observed in invasive carcinoma [13].

^{111}In -DTPA-hEGF injection

^{111}In -DTPA-hEGF was prepared from a kit manufactured at the University Health Network radiopharmacy [9]. Each kit contained DTPA-hEGF (0.25 mg) in 1.0 mL sodium acetate buffer (1 M, pH 6.0). ^{111}In -DTPA-hEGF was prepared by adding 370-2,220 MBq of ^{111}In chloride (MDS-Nordion, Kanata, ON) and incubating at room temperature for 30 min. The final radiopharmaceutical was obtained by diluting ^{111}In -DTPA-hEGF with Sodium Chloride Injection USP and filtration through a 0.22 μm Millex-GV (EMD Millipore, Billerica, MA) filter. Radiochemical purity was assayed using instant thin layer-silica gel (ITLC-SG) chromatography as previously described [9] and >90% was required for clinical use.

Imaging and radiation dosimetry

Imaging was performed using a dual-detector gamma camera fitted with medium energy, general purpose collimators (Cobra II® Series Auto-Gamma System Model 5003, Packard Instrument Company, Meriden, CT, USA). Two 20% windows, to include the gamma photon peaks of ^{111}In (173 and 247 keV) were used. Whole-body planar images were acquired at 1, 4-6, 24 and 72 h p.i. SPECT images were acquired to evaluate radiopharmaceutical uptake at known sites of disease. SPECT was performed at 24 and 72 h for the first 5 patients to determine the optimal time point for imaging and performed for other study participants at 24 h only. Interpretation of ^{111}In -DTPA-hEGF scintigraphy was performed in the presence of CT images (showing sites of suspected tumors). ^{111}In -DTPA-hEGF scintigraphy was recorded as positive by study radiologists (M.F., I.C.) when radiopharmaceutical uptake was greater in at least one metastatic site (based on correlative CT scans) compared to the background for the corresponding region.

Radioactivity in the syringe containing ^{111}In -DTPA-hEGF was measured in a dose calibrator (Capintec CRC25R, Ramsay, NJ) and, following injection, the syringe was re-measured. The difference between the two measurements

Phase I trial of ¹¹¹In-DTPA-hEGF

Table 1. Patients

Patient No.	Age (yr)	No. of prior courses of systemic therapy		ER [†]	PR [†]	Known sites of metastases [§]	Dose of ¹¹¹ In-DTPA-hEGF (MBq) [‡]
		Chemotherapy	Hormone therapy				
1	44	4	0	N	N	Li, Lu, B, Br	408
2	42	9	1	P	P	Li, Lu, B	382
3	51	4	0	N	N	CW, LN	406
4	44	6	0	N	N	B	406
5	50	5	0	N	N	Lu	434
6	59	6	3	P	UK	Li, Lu, B	378
7	51	3	5	P	P	B	357
8	58	4	0	N	N	LN	805
9	48	4	4	N	P	B, LN	790
10	46	6	2	P	P	SC	754
11	52	3	1	N	N	Lu, LN	1,527
12	56	4	0	N	N	Lu, B	1,408
13	43	2	0	N	N	SC	1,241
14	35	3	0	N	N	Lu, B	2,030
15	46	6	1	N	N	Sk, LN, Lu	2,290
16	46	2	1	N	P	Lu, SC	2,195

*ER, Estrogen receptor status of primary tumour. †PR, Progesterone receptor status of primary tumour. N, negative; P, positive; UK, unknown. §Li, liver; Lu, lung; B, bone; Br, brain; CW, chest wall, LN, lymph nodes; SC, subcutaneous; Sk, skin. ‡The mass of DTPA-hEGF was 0.25 mg for all radioactivity doses.

was the administered amount of ¹¹¹In. Conjugate view whole-body images were analyzed to obtain the counts in regions-of-interest (ROIs) defining normal organs. The geometric mean counts per minute in the ROIs from anterior and posterior views were calculated and corrected for tissue attenuation using an attenuation map. The program used to extract the volume and count data was ADAC Pegasys™X, Version 4.2 (Philips Healthcare, Best, Netherlands). The attenuation map was calculated by obtaining an anterior image of the patient with a homogeneous ⁵⁷Co flood source (370 MBq) suspended beneath the couch. Attenuation for the ⁵⁷Co flood was converted to the expected attenuation for ¹¹¹In using a previously developed calibration curve. The ratio of counts obtained in each pixel with and without the patient on the bed was used to derive tissue attenuation correction factors. Pixel counts, once corrected for attenuation and scatter, were converted to radioactivity values (kBq) using a conversion factor based on imaging a point source of ¹¹¹In. The radioactivity (kBq) in organs at each imaging time point was plotted versus time p.i. using Prism® Version 4.0 software (Graphpad, San Diego, CA). The area under the curve (AUC) from time zero to the final time point (kBq × h) was calculated. The

mean residence time for ¹¹¹In (τ, h) in each organ was calculated by dividing the total AUC (kBq × h) by the injected radioactivity (kBq). Radiation dosimetry estimates were calculated using the OLINDA/EXM program.

Pharmacokinetics

Blood was collected at baseline, 5, 15, 30 minutes and 1, 2, 4-6, 24, 48 and 72 h p.i. A 1.0 mL sample of whole blood and plasma at each time was dispensed in duplicate into gamma counting tubes and the contained radioactivity, along with a standard of the radiopharmaceutical, was measured in a gamma scintillation counter (Cobra II® Series Auto-Gamma® Counting System, Packard Instrument Co., Meriden, CT, USA). Counts were converted to kBq/mL and blood or plasma radioactivity plotted versus time using Prism® Version 4.0. The curve was fitted to a one- or two-compartment pharmacokinetic model by Scientist Ver. 2.0 (Micromath, St. Louis, MO). Pharmacokinetic parameters ($t_{1/2\alpha}$, $t_{1/2\beta}$, V_1 , V_{ss} , CL) were calculated. For participants receiving the highest administered amount (2,220 MBq), samples at 24, 48 and 72 h p.i. only were available. A 24-h urine collection starting immediately p.i. was obtained. The percentage of the injected radio-

Table 2. Adverse Events and Laboratory Toxicities of All Grades*

Toxicity	Number of patients		All	
	Grade 1	Grade 2	No	%
Elevated AST or ALT [†]	2	–	2	13
Anemia	1	–	1	7
Hypotension	1	2	3	20
Nausea	6	6	12	80
Vomiting	4	4	8	53
Heartburn	1	–	1	7
Flatulence	–	1	1	7
Dysphagia	1	–	1	7
Dry Mouth	4	4	8	53
Taste disturbance	5	–	5	33
Flushing	14	–	14	93
Chills	8	2	10	67
Fatigue	6	1	7	47
Diaphoresis	1	–	1	7
Fever	–	1	1	7
Headache	4	3	7	47
Pain	6	1	7	47
Dry eye	–	2	2	13
Pruritus	3	–	3	20
Paraesthesia	5	–	5	33
Tremor	–	1	1	7
Dizziness	5	3	8	47
Vision-flashing	1	–	1	7

*This table includes adverse events that were considered to be 'possibly', 'probably' or 'definitely' related to ¹¹¹In-DTPA-hEGF (according to NCI-CTCv2 attribution standards). Common Terminology Criteria for Adverse Events (CTCAE) v2 was used to score toxicity [10]. [†]AST or ALT: Aspartate aminotransferase or Alanine aminotransferase.

activity eliminated in the urine in 24 h was calculated.

Immunogenicity

Testing for anti-DTPA-hEGF antibodies was performed by ELISA on serum samples obtained before administration of ¹¹¹In-DTPA-hEGF and at 2, 4 and 12 weeks p.i. Wells of a microplate were coated for 1 h at 37°C with 50 µL of DTPA-hEGF (10 µg/mL in 50 mM sodium bicarbonate buffer, pH 7.5) and rinsed with 0.5% (v/v) Tween-80 in Phosphate-Buffered Saline, pH 7.4 (Tween-80/PBS). Serum samples (200 µL) (diluted 1:100 to 1:10,000 in Tween-80/PBS) were added. Following incubation for 1 h at 37°C, wells were rinsed with Tween-80/PBS,

then incubated for 1 h at 37°C with 200 µL of goat anti-human IgG (Fc-specific) antibodies conjugated to horseradish peroxidase (1:15,000 dilution). Following rinsing with Tween-80/PBS, 200 µL of o-phenylenediamine dihydrochloride solution and H₂O₂ were added and allowed to react for 30 mins. The color produced was measured in a ELx800 multi-well plate reader (Bio Tek Instruments Inc., Winooski, VT, USA) at 450 nm with subtraction of background at 690 nm (Abs₄₅₀₋₆₉₀).

Statistical analysis

Descriptive statistics were used to summarize patient characteristics, immunogenicity, dosimetry and biodistribution. All reported *P*-values were 2-sided and *P*≤0.05 was considered significant. SASv9.1 software (SAS Institute, Cary, NC) was used.

Results

Study participants

Sixteen women (median age 47 yr, range 35-59) with metastatic EGFR-positive breast cancer received ¹¹¹In-DTPA-hEGF (**Table 1**). All had received between 2 and 10 prior regimens of systemic treatment for metastatic disease (median, 4.4). The number of study participants that had previously received a regimen containing an anthracycline, taxol or trastuzumab (Herceptin) was 15, 12 and 10, respectively. No patient received prior EGFR-targeted therapy. ECOG performance status at trial entry was 0, 1 or 2 in 9, 5 and 2 patients, respectively. Seven patients were recruited to the first cohort and three patients to each subsequent cohort.

Toxicity and antitumor activity

One study participant in the first cohort died within 6 weeks of treatment with ¹¹¹In-DTPA-hEGF from progressive disease (PD) and was unevaluable for toxicity. An additional patient was enrolled. Another member of the first cohort developed Grade 3 thrombocytopenia. She had extensive bone marrow involvement by cancer and thrombocytopenia was thought unlikely to be related to ¹¹¹In-DTPA-hEGF (CTCAEv2.0 attribution standards) [10]. However, this event was classed as a DLT and the first cohort was expanded by 3 subjects. A summary of AEs is shown (**Table 2**). The common-

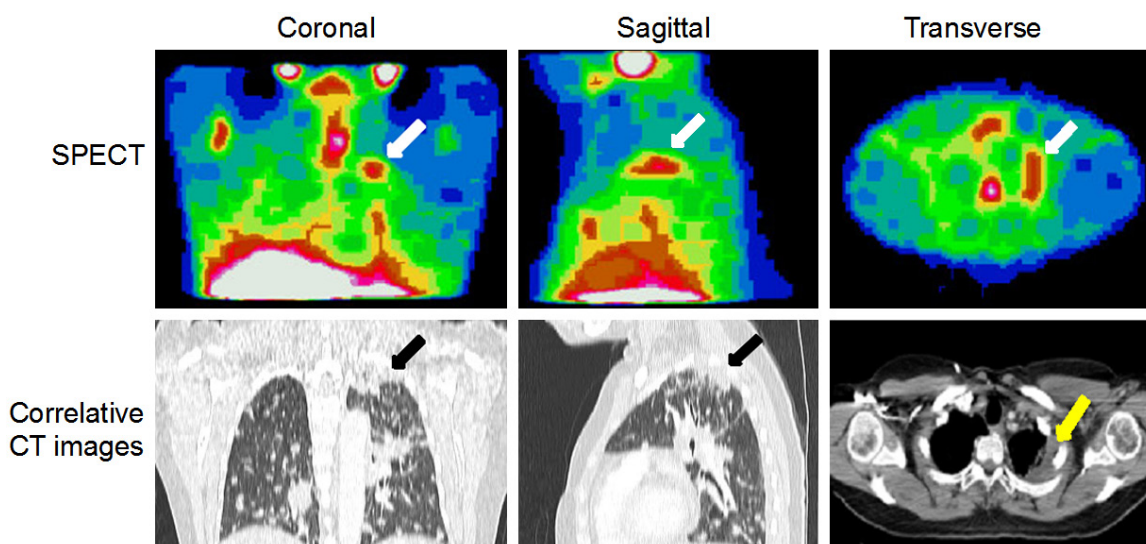


Figure 1. Representative images from Patient 11 showing tumor accumulation of ^{111}In -DTPA-hEGF with correlative CT images. Coronal, sagittal and transverse SPECT images at 24 h p.i. A tumor deposit present in the left lung apex (arrows) shows ^{111}In uptake.

est AEs were flushing, chills, nausea and vomiting that occurred transiently during and after ^{111}In -DTPA-hEGF administration. No non-hematological Grade 3 or 4 AEs occurred. Two patients experienced Grade 2 hypotension. Hepatic transaminases were elevated (Grade 1) in 2 patients at up to 180 days p.i. This hepatic dysfunction was observed at the time of PD. Serum Cr was normal for all patients throughout the trial. One patient experienced Grade 1 anemia. None experienced a fall in neutrophil count below the normal range. There was no clear relationship between the severity of toxicity and the amount of radioactivity administered. For example, the patients who experienced Grade 2 hypotension were members of the first and second cohorts. There were 4 episodes of Grade 2 vomiting and these were experienced by one patient from each of the cohorts.

Stable disease (SD) was observed in 4 (27%) patients (two from dose level one and one each from dose levels 2 and 3). The mean duration of SD was 5.8 months. No objective responses (i.e. complete [CR] or partial response [PR]) were observed in 15 evaluable patients determined using RECIST criteria. Tumor response was a secondary aim in this first-in-human study of ^{111}In -DTPA-hEGF. The primary aims of the study were to determine the pharmacokinetics, toxicity profile and biodistribution of ^{111}In -DTPA-hEGF.

Imaging, radiation dosimetry, pharmacokinetics and immunogenicity

There was accumulation of radioactivity in at least one known site of disease in either the whole-body planar or SPECT images in 7 of 15 evaluable patients (47%) and slight uptake in at least one known site of disease in a further 3 patients (20%). ^{111}In -DTPA-hEGF positive lesions were observed in lung, bone, subcutaneous/skin and lymph node metastases. Sensitivity for liver metastases was poor, possibly because of high hepatic background, particularly at early time points. The number of study participants with clearly positive scans per number of evaluable patients in cohorts 1, 2, 3, and 4 were 3/6, 0/3, 2/3 and 2/3, respectively. Thus, there was no clear relationship between the amount of injected radioactivity and visualization of tumor uptake of ^{111}In -DTPA-hEGF. Representative SPECT images showing tumor accumulation, with correlative CT images are shown (Figures 1 and 2).

Normal tissue accumulation of ^{111}In -DTPA-hEGF was mainly in the liver (30-40%), kidneys (10-15%), spleen (1.5-2%) and thyroid (<1%). Mean organ dose estimates are shown in Table 3. For the highest radioactivity level administered (2,200 MBq) the radiation absorbed doses for whole body, liver, kidneys, spleen and thyroid were 0.18, 1.91, 1.62, 0.82, 0.67 Gy, respectively. ^{111}In -DTPA-hEGF was cleared biexponentially.

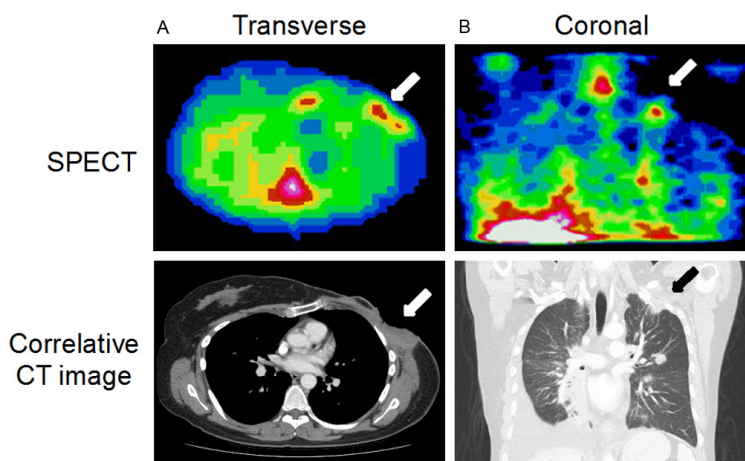


Figure 2. Representative images from two patients showing tumor accumulation of ¹¹¹In-DTPA-hEGF with correlative CT images. A: Transverse SPECT image (Patient 13) acquired 24 h p.i. showing accumulation of ¹¹¹In in an area of chest wall disease (arrow). B: Shows a coronal SPECT image (Patient 15), acquired 24 h p.i. demonstrating accumulation of radioactivity in a left infraclavicular fossa and lung apex tumor deposit.

Table 3. Mean Organ Radiation Absorbed Dose Estimates

Organ	Mean Dose \pm SD (mGy/MBq)
Adrenals	0.20 \pm 0.01
Brain	0.02 \pm <0.01
Breasts	0.04 \pm <0.01
Gallbladder	0.27 \pm 0.02
Heart	0.10 \pm 0.01
Kidneys	0.74 \pm 0.14
Liver	0.86 \pm 0.10
Lower large intestine	0.05 \pm 0.01
Lungs	0.09 \pm 0.01
Muscle	0.05 \pm <0.01
Bone	0.10 \pm 0.01
Ovaries	0.05 \pm <0.01
Pancreas	0.18 \pm 0.01
Bone marrow	0.06 \pm <0.01
Skin	0.03 \pm <0.01
Small intestine	0.08 \pm 0.01
Spleen	0.37 \pm 0.10
Stomach	0.10 \pm 0.01
Thymus	0.05 \pm <0.01
Thyroid	0.30 \pm 0.15
Upper large intestine	0.10 \pm 0.01
Urinary bladder	0.17 \pm 0.06
Uterus	0.06 \pm 0.01
Total body	0.08 \pm 0.01

tially from the blood with an α -phase $T_{1/2}$ of 0.16 \pm 0.03 h and β -phase $T_{1/2}$ of 9.41 \pm 1.93 h. A

representative elimination curve is shown (Figure 3). Pharmacokinetic parameters for ¹¹¹In-DTPA-hEGF are shown in Table 4. The 24 h urinary excretion of radioactivity was 16.05 \pm 2.45% (mean \pm SEM). Although the risk of antibody formation against human EGF was predicted to be low, it was considered prudent to test for it, as the addition of a chelator (DTPA) could alter immunogenicity. However, there was no evidence of immunogenicity since the logarithm of the titer for binding of serum samples to DTPA-hEGF in a microELISA plate and detected with goat anti-human IgG secondary antibodies did not increase (up to 3 months p.i.). These values did not significantly differ from unity and were 0.97 \pm 0.08, 1.08 \pm 0.12 and 1.09 \pm 0.13 for <1, 1 and 3 months p.i.

Discussion

There is growing recognition that molecularly-targeted radiopharmaceuticals that incorporate Auger electron-emitting radionuclides can provide a precise means of delivering lethal radiation doses to cancer cells. However, to be effective, Auger electron-emitters should accumulate specifically in the nucleus [14]. Strategies for achieving this include linkage of cell-penetrating peptides, nuclear localizing sequences (NLS) or both [15], to radiolabeled carrier molecules in order to enhance nuclear uptake or the use of radiolabeled triplex-forming oligonucleotides that deliver radiation to specific genes [16]. Another approach is to take advantage of a peptide ligand that accumulates in the nucleus. This is the principle behind the design of ¹¹¹In-DTPA-hEGF, which binds the EGFR. The receptor-ligand complex, through a NLS in the juxtamembrane region of EGFR, translocates to the nucleus, coming into close proximity with DNA [5].

Although Auger electron radiation represents an attractive alternative to higher energy β -particle therapy, this approach has only been tested in a small number of clinical studies to date. ¹¹¹In-DTPA-octreotide (¹¹¹In-pentetreotide) was tested in a Phase I trial of 50 patients with

Phase I trial of ^{111}In -DTPA-hEGF

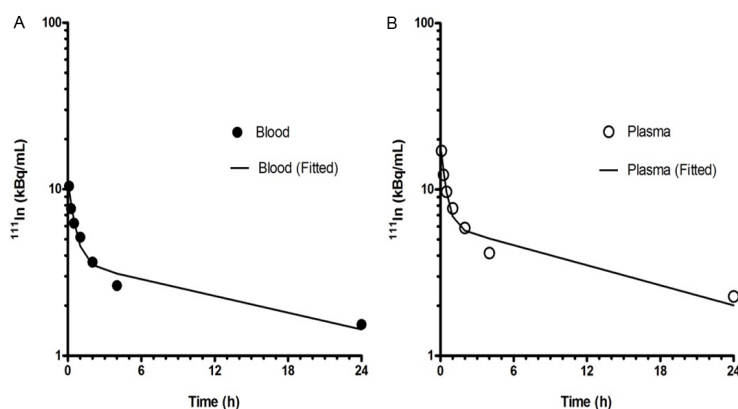


Figure 3. Curves showing representative biphasic elimination of radioactivity from (A) blood and (B) plasma following administration of ^{111}In -DTPA-hEGF (Patient 8). In this patient the $T_{1/2\alpha}$ and $T_{1/2\beta}$ in the blood were 0.35 and 17.9 h, respectively while the $T_{1/2\alpha}$ and $T_{1/2\beta}$ in the plasma were 0.28 and 14.9 h, respectively.

Table 4. Pharmacokinetic Parameters for Elimination of ^{111}In -DTPA-hEGF

Parameter	Blood	Plasma
	Mean \pm SEM	
V_1 (L/kg)	0.46 \pm 0.11	0.49 \pm 0.12
V_{ss} (L/kg)	2.64 \pm 0.73	5.68 \pm 3.81
CL (L/h/kg)	0.27 \pm 0.06	0.21 \pm 0.06
$T_{1/2\alpha}$ (h)	0.16 \pm 0.03	0.16 \pm 0.03
$T_{1/2\beta}$ (h)	9.41 \pm 1.93	12.19 \pm 3.70
k_{21} (h^{-1})	0.90 \pm 0.16	1.77 \pm 0.44
k_{10} (h^{-1})	1.91 \pm 0.62	3.06 \pm 1.05
k_{12} (h^{-1})	4.62 \pm 1.24	6.04 \pm 2.11

V_1 : volume of distribution; V_{ss} : volume of distribution at steady-state; CL: systemic clearance; $T_{1/2\alpha}$: alpha-phase half-life; $T_{1/2\beta}$: beta phase half-life; k_{21} : microconstant for transfer from compartment 2 to 1; k_{10} : microconstant for elimination from compartment 1; k_{12} : microconstant for transfer from compartment 1 to 2.

neuroendocrine tumors who received 20-160 GBq [17]. Of 40 evaluable patients, 20 experienced SD or minor response (25-50% tumor volume reduction). Bone marrow toxicity was common and usually mild although 3 patients, who received >100 GBq, developed myelodysplastic syndrome or leukemia. In another trial, 26 patients with somatostatin receptor-positive malignancies received two doses of ^{111}In -pentetreotide of 6.6 GBq each [18]; PR was observed in two patients. These results indicate that objective tumor responses are unusual with ^{111}In -labeled somatostatin analogues. This may be because the uptake of

^{111}In -DTPA-octreotide into cell nuclei appears to be modest [19]. Auger electron-emitting radiopharmaceuticals such as ^{111}In -DTPA-hEGF, that are specifically designed to accumulate in the nucleus, have the potential to be effective due to the 30-fold higher radiation absorbed dose delivered to the nucleus from ^{111}In in the nucleus compared to the cytoplasm [20]. In a case report of a patient with advanced pancreatic cancer with meningeal metastases a combination of intrathecal 5-iodo-2'-deoxyuridine (^{125}I UdR) plus methotrexate resulted in a fall in CA 19-9 and clinical improvement [21]. Thus the current report of the first-in-

human trial of ^{111}In -DTPA-hEGF contributes new knowledge to an under-researched area, particularly the feasibility of targeted Auger electron therapy of tumors in patients.

In pre-clinical studies, mice received a total of 27.7, 55.5 or 92.5 MBq ^{111}In -DTPA-hEGF divided over 5 weekly doses. Significant anti-tumor effects were observed at 7 weeks post-treatment even in the lowest dose group [8]. These preclinical studies established proof-of-principle for the treatment of EGFR-amplified tumors with ^{111}In -DTPA-hEGF. As the current study was a first-in-human trial of an investigational agent, the lowest dose of ^{111}In -DTPA-hEGF selected (370 MBq) was guided by the clinical experience with ^{111}In -pentetreotide used for diagnostic imaging which is known to be safe. The highest dose administered was selected on the basis that this fell within in the range safely administered to rabbits and mice in pre-clinical toxicology studies [4]. In addition, this dose range was restricted by Health Canada approval of the CTA.

Consistent with pre-clinical toxicology studies, ^{111}In -DTPA-hEGF did not cause perturbation of hematological, renal or hepatic function [4]. Myelosuppression is often a DLT of β -emitting radiopharmaceuticals [22]. In contrast the estimated radiation absorbed dose to the bone marrow after ^{111}In -DTPA-hEGF was low (0.06 mGy/MBq) and no bone marrow toxicity was observed. One possible explanation for this is the low EGFR expression of haemopoietic lineages, which would limit internalization and

cytotoxicity of ^{111}In -DTPA-hEGF [23]. EGFR is expressed to moderate levels in renal and hepatic cells but no adverse effect of ^{111}In -DTPA-hEGF on these organs was observed [24]. The mean radiation absorbed dose to kidneys and liver was 0.74 and 0.86 mGy/MBq, respectively. Thus, following the highest administered dose of ^{111}In -DTPA-hEGF (2,200 MBq), the radiation absorbed doses to the kidneys and liver were 1.62 and 1.91 Gy, respectively. Doses of 23 Gy to the kidney and 30 Gy to the liver delivered using external beam radiation therapy would be expected to cause significant toxicity in approximately 5% of individuals [25]. The radiation absorbed dose to these organs following treatment with ^{111}In -DTPA-hEGF, therefore suggests dose escalation would be possible. However, the organ and whole body doses (Table 3) were calculated using a macrodosimetry method and do not account for cellular and subcellular distribution of the radionuclide which could result in dose heterogeneities, making the probability of toxicity difficult to predict [26]. The lack of renal toxicity observed in patients who received ^{111}In -DTPA-hEGF is in contrast to several other radiopeptides, such as ^{90}Y - or ^{177}Lu -DOTATOC [27]. Radiation toxicity depends on microdosimetry and particularly the radiation dose deposited in the glomeruli which are radiosensitive. ^{111}In -pentetreotide resulted in heterogeneous dose distribution with low dose deposition in the glomeruli [26]. Even following high-activity ^{111}In -pentetreotide therapy (with cumulative administered amounts as high as 36.6 Gy), renal toxicity was not observed [28]. It is possible that ^{111}In -DTPA-hEGF has a similar dose distribution accounting for its lack of renal toxicity.

The amount of DTPA-hEGF administered was 0.25 mg (approximately 5 $\mu\text{g}/\text{kg}$) and was much lower than the equivalent maximum amount administered to mice (1,500 mg/kg) in toxicology studies [4]. An observed side-effect following ^{111}In -DTPA-hEGF was transitory flushing and 2 patients experienced Grade 2 hypotension. Hypotension was observed previously in primates receiving EGF, suggesting that the vasodilatory effect of ^{111}In -DTPA-hEGF is due to the EGF moiety. For example, monkeys given 3 or 300 $\mu\text{g}/\text{kg}$ EGF as a 20 min infusion experienced a fall in blood pressure of up to 20% and 32%, respectively [29]. In humans ^{131}I -labeled EGF (0.3 mg) was administered to patients with lung cancer [6]. Some patients, who received

up to 2.7 mg of co-infused non-labeled EGF (giving a total EGF dose of 3 mg), experienced AEs similar to those observed with ^{111}In -DTPA-hEGF: nausea, vomiting and chills. The dose of EGF used in the current trial was selected to allow consistently high radiolabeling efficiency (>90%) and to avoid the need for post-labeling purification at high amounts of ^{111}In . ^{111}In -DTPA-hEGF exhibited biexponential clearance, with α -phase $T_{1/2}$ of 0.16 ± 0.03 h and β -phase $T_{1/2}$ of 9.41 ± 1.93 h (Table 4). This is consistent with results for ^{131}I -labeled-EGF in lung cancer patients, in whom α -phase $T_{1/2}$ was 0.84 ± 0.40 h and the β -phase $T_{1/2}$ was 15.2 ± 1.4 h following 0.3 mg EGF [6].

The moderately high expression level of EGFR in hepatocytes could result in ligand accumulation in the liver, limiting the amount available for tumor uptake. Others have sought to overcome liver uptake of radiolabeled anti-EGFR antibodies by increasing the mass dose [30]. However this strategy was not feasible in the current study due to the vasodilatory effect of EGF noted at a mass dose of 0.25 mg. Thus the biological activity of native EGF is a limitation. However modified forms of the peptide that have EGFR blocking activity have recently been reported [31]. It is possible that these will avoid some adverse biological effects of the native peptide and be useful for targeted radiotherapy.

Antitumor response was not a defined endpoint in this Phase I trial. However, tumor size was evaluated using RECIST. There were no objective responses. The lack of objective response to ^{111}In -DTPA-hEGF could be due to low or heterogeneous expression of EGFR in the tumors of some study participants, as it has been shown that ^{111}In -DTPA-hEGF is only cytotoxic in cells with $\geq 1.3 \times 10^5$ EGFR [32]. In many studies of EGFR immunohistochemistry, tumors are regarded as positive if any level of staining is observed [13]. This approach was taken in the current study, so patients whose tumors exhibited only modest EGFR expression were eligible. It is possible that in cases with low or heterogeneous EGFR expression, there was insufficient accumulation of ^{111}In -DTPA-hEGF to produce an antitumor effect. In the future it would be prudent to select for ^{111}In -DTPA-hEGF treatment only patients whose tumors are highly EGFR-amplified. Concordance between EGFR in primary breast cancers and their

metastases has been reported [33]. Expression of EGFR has been observed to be higher in metastases compared to corresponding primary tumors more frequently than the reverse [34]. Using the EGFR protein expression level of the primary tumor as the basis for selection for treatment with ^{111}In -DTPA-hEGF therefore seems reasonable based on current evidence.

Maximum tolerated dose (MTD) was not reached in this trial. To increase the administered amount of radioactivity above 2,200 MBq would have necessitated increasing the mass amount of hEGF above 0.25 mg. This was considered undesirable because flushing, nausea/vomiting and hypotension were thought to be attributable to EGF. However, imaging showed that tumor localization was achieved and since there were no serious adverse effects, it is reasonable to consider administering higher doses of radioactivity through multiple administrations of doses that were found to be safe. Therefore, a decision was made to stop this trial and plan to increase the administered amount of ^{111}In -DTPA-hEGF, through repeat dosing, in a future trial. Anti-DTPA-hEGF antibodies were not detected, therefore evaluation of ^{111}In -DTPA-hEGF in a multi-dose schedule is unlikely to result in an immunological response and should be feasible.

Conclusion

^{111}In -DTPA-hEGF is safe when given as a single dose up to 2,300 MBq (0.25 mg). It is cleared bioexponentially from the blood and excreted predominantly in the urine. Normal organs which accumulate ^{111}In -DTPA-hEGF include the liver and kidneys, but mean radiation absorbed dose estimates were within accepted tolerance. ^{111}In -DTPA-hEGF localized in EGFR-positive lesions in many patients even when a broad definition of EGFR positivity was used. There was no evidence of significant treatment-associated toxicity at the doses studied during median follow-up of 8.3 months. The safety profile, favourable radiation dosimetric data and lack of immunogenicity indicate that further dose escalation of ^{111}In -DTPA-hEGF is feasible.

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Address correspondence to: Katherine A Vallis, Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, OX3 7LJ. Tel: +44 (0)1865255850; Fax: +44 (0)1865857533; E-mail: katherine.vallis@oncology.ox.ac.uk; Raymond M Reilly, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada M5S 3M2. Tel: 1-416-946-5522; Fax: 1-416-978-8511; E-mail: raymond.reilly@utoronto.ca

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