A Phase I Clinical Trial of CHT-25 a ^{131}I-Labeled Chimeric Anti-CD25 Antibody Showing Efficacy in Patients with Refractory Lymphoma

Gairin Dancey,[1] John Violet,[1] Alessandra Malaroda,[1] Alan J. Green,[1] Surinder K. Sharma,[1] Roslyn Francis,[1] Shokri Othman,[1] Sweta Parker,[1] John Buscombe,[3] Natalie Griffin,[1] Pei-San Chan,[3] Anmol Malhotra,[3] Nicholas Woodward,[3] Alan Ramsay,[2] Philip Ross,[7] T. Andrew Lister,[6] Peter Amlot,[4] Richard Begent,[1] and Christopher McNamara[5]

Abstract

Purpose: There is a need for new treatments for Hodgkin and T-cell lymphoma due to the development of drug resistance in a proportion of patients. This phase I study of radioimmunotherapy used CHT-25, a chimeric antibody to the α-chain of the interleukin-2 receptor, CD25, conjugated to iodine-131 (^{131}I) in patients with refractory CD25-positive lymphomas.

Experimental Design: Fifteen patients were treated (Hodgkin lymphoma, 12; angioimmunoblastic T-cell lymphoma, 1; adult T-cell leukemia/lymphoma, 2). Tumor was monitored by computed tomography and in all but two patients by ^{18}F-fluorodeoxyglucose positron emission tomography.

Results: There were no grade 3 or 4 infusion reactions. At the maximum tolerated dose of 1,200 MBq/m^2, the major side effect was delayed myelotoxicity with the nadir for platelets at 38 days and for neutrophils at 53 days. One patient treated with 2,960 MBq/m^2 developed prolonged grade 4 neutropenia and thrombocytopenia and died of Pneumocystis jiroveci pneumonia. Nonhematologic toxicity was mild. Single photon emission computer tomography imaging showed tumor-specific uptake and retention of ^{131}I and no excessive retention in normal organs. Of nine patients receiving ≥1,200 MBq/m^2, six responded (three complete response and three partial response); one of six patients with administered radioactivity of ≤740 MBq/m^2 had a complete response.

Conclusions: CHT-25 is well tolerated with 1,200 MBq/m^2 administered radioactivity and shows clinical activity in patients who are refractory to conventional therapies. Phase II studies are justified to determine efficacy and toxicity in a broader range of clinical scenarios. (Clin Cancer Res 2009;15(24):7701–10)

In spite of recent advances in therapy, there remains an acute need for improved treatments for Hodgkin lymphoma (HL) and other lymphomas. For instance, there is only a 50% prospect of long-term disease free survival for patients with relapsed HL responding to high-dose chemotherapy and autologous stem cell transplant (1, 2). The interleukin-2 (IL-2) receptor is selectively overexpressed in HL and other lymphomas and therefore is a potential target for new therapies. Radioimmunotherapy (RIT) with CHT-25 [Iodine-131 (^{131}I)–labeled chimeric antibody to the IL-2 receptor (CD25)] has been developed for this purpose and a phase I study in relapsed HL and T-cell lymphoma is reported.

CD25 is a 55-kDa[3] protein with three transmembrane protein chains that is induced on T-cell activation but is not found on B or T lymphocytes or monocytes in the resting state (3, 4). Hodgkin/Reed-Sternberg cells frequently express CD25, and the

Authors' Affiliations: [1]Cancer Research UK Targeting and Imaging Group, Department of Oncology, University College London Cancer Institute and [2]Department of Histopathology, University College London Hospitals, London, United Kingdom; Departments of [3]Nuclear Medicine and Radiology, [4]Immunology, and [5]Hematology, Royal Free Hospital, Hampstead, United Kingdom; [6]Cancer Research UK Medical Oncology Unit, St Bartholomew's Hospital, London, United Kingdom; [7]Drug Development Office, Cancer Research UK, London, United Kingdom

Received 6/3/09; revised 9/2/09; accepted 9/5/09; published online 12/15/09.

Grant support: Cancer Research UK grant C34/A5149. The trial was sponsored by Cancer Research UK through the Drug Development Office. The work was also supported by the University College London Experimental Cancer Medicine Centre and University College London Cancer Institute Research Trust. This work was undertaken at University College London which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centers funding scheme. We thank the Mark Fisher Trust and the James Baldwin Trust for additional support. Initial supply of the anti-CD25 antibody, basiliximab, was provided by Novartis. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Preliminary clinical response and toxicity data were presented at the 49th annual meeting of the American Society of Hematology, Atlanta. The clinical trial and report is the original work of the authors.

Requests for reprints: Richard Begent, Department of Oncology, University College London Cancer Institute, Paul O'Gorman Building, Huntley Street, London WC1E 6BT, United Kingdom. Phone: 44-20-7679-0706; Fax: 44-20-3108-2025; E-mail: r.begent@ucl.ac.uk.

doi:10.1158/1078-0432.CCR-09-1421

Translational Relevance

A clinical trial is reported showing substantial therapeutic effects from radioimmunotherapy in Hodgkin and T-cell lymphomas using 131-Iodine–labeled CHT25 chimeric antibody against the interleukin-2 receptor. The chimeric mouse/human antibody did not generally generate an antiantibody response; as a result, it was possible to deliver repeated therapy. CHT25 cleared slowly from the circulation delivering therapy to tumor deposits over a sustained period. Effective therapy could thus be achieved without excessive toxicity at the maximum tolerated dose. Preliminary pharmacokinetic and pharmacodynamic information is reported on the conditions needed for safe and effective therapy. Potential advantages of chimeric antibodies in radioimmunotherapy have been identified, which may be applicable in common epithelial tumors as well as in lymphoma. Phase II clinical trials are being developed to determine efficacy of CHT25 in defined clinical situations either as a single agent or combined with chemotherapy.

well vascularized nature of lymphoma tissue makes it accessible to i.v. administered antibodies (5, 6).

Although therapy with unlabeled antibody is effective for many B-cell lymphomas, (7, 8), it has shown limited activity in HL (9) and only short-term benefit in human T-cell lymphotrophic virus–associated lymphoma/leukemia where the IL-2 receptor is part of an important growth pathway (10). The problem of low potency can be addressed by conjugation of antibody to immunotoxins (11, 12) or by radiolabeling with a radionuclide such as ^{131}I or Yttrium-90 (^{90}Y; ref. 13).

Repeated treatment is often compromised with murine antibodies due to their immunogenicity(14, 15). We have addressed this by using a chimeric antibody with mouse variable regions (murine RFT5 cell antibody) and human constant regions with proven low immunogenicity (16–18). The antibody binds with an affinity approximating that of IL-2 itself (17) and in its unlabeled form is licensed for the prevention of acute cellular rejection in renal allografts (18). It is known to have a longer blood half-life than the murine antibodies directed against the same epitope (17) and differs in this respect from the murine antibodies ibritumomab and tositumomab also used in RIT (19–21).

This article is believed to be the first to describe a clinical trial investigating the safety and efficacy of RIT with CHT-25 in refractory CD25-positive HL and other CD25-positive lymphomas.

Patients and Methods

The study was approved by the local Research Ethics Committee and the U.K. Medicines and Healthcare Products Regulatory Agency. All patients provided written informed consent. Patients with histologically confirmed lymphoma expressing CD25 on >50% of tumor cells (or the surrounding tumor milieu in HL) from a biopsy at diagnosis or relapse were eligible. Other inclusion criteria included the

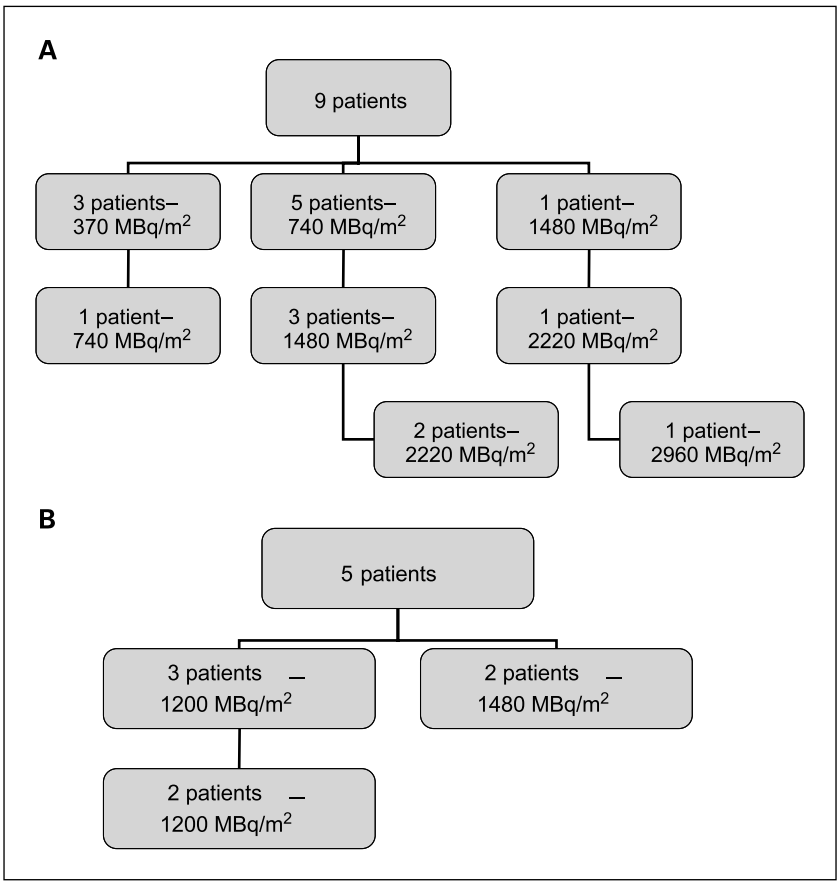

Fig. 1. *A,* escalation phase from 370 to 2,960 Mbq/m^2: includes the first nine patients treated only. *B,* MTD finding phase: the second phase of the study to refine the dose, includes patient 10 to 14 (patient 15 excluded).

following: lymphoma refractory to prior therapy or relapsed after standard chemotherapy, age 18 y or older, measurable disease by computed tomography (CT), life expectancy of ≥3 mo, no serious comorbidities including HIV infection, WHO performance status of ≤2, bone marrow examination within 4 wk showing ≤25% involvement by tumor with adequate normal hematopoiesis, glomerular filtration rate of >50 mL/min, left ventricular ejection fraction on a multiple gated acquisition scan of >50%, absolute neutrophil count of ≥1.5 × 10^9/liter hemoglobin of ≥10 g/dl, platelet count of ≥100 × 10^9/liter, plasma creatinine mol/L, aspartate aminotransferase/alanine aminotransferase of ≤150 mol/L or EDTA clearance ≥50 mL/min, plasma bilirubin of ≤30 2× upper limit of normal, international normalized ratio of prothrombin time (INR) of ≤1.5, and normal thyroid function or stable on treatment. All patients were human anti-mouse antibody negative before therapy and any chemotherapy or radiotherapy was completed at least 4 wk before CHT-25 administration; corticosteroids were permitted as long as the dose was unchanged in the previous 4 wk.

Study design. This trial was an open-label, nonrandomized, phase I, multiple dose escalation study. Primary objectives were to determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT) and pharmacokinetic analysis. Secondary objectives were tumor response assessment and immunogenicity.

Dose escalation and major toxicity rules. An accelerated titration design was used initially with stem cell support offered at doses that may be myeloablative (≥1,480 MBq/m^2). A 100% dose escalation was used initially with three patients per cohort until 1,480 MBq/m^2 with the next increment at 50% and the final at 30% increase. The dose groups were as follows: 370, 740, 1,480, 2,220, and 2,960 MBq/m^2. The starting dose of 370 Mb/m^2 was chosen based on medical internal radiation dose (MIRD) dosimetry calculations from a previous study suggesting an absence of significant toxicity with a chimeric antibody. In the initial, possibly myeloablative, dose escalation phase, patients could be retreated if all drug-related toxicities (excluding hematological) had resolved to grade 2 or less. In the MTD finding phase, all toxicity (including hematological) had to recover to grade 2 or less, with a neutrophil count of >1.0 × 10^9/L and a platelet count of >100 × 10^9/L.

Escalation was stopped after the first DLT occurred at 2,960 MBq/m^2, which led to the only treatment-related death. Evidence of response had already been shown above 740 MBq/m^2 with stem cell support only required above 1,480 MBq/m^2. A further cohort was therefore included midway between 740 and 1,480 MBq/m^2 (at 1,200 MBq/m^2) to exploit this combination of response together with no requirement for stem cell storage. The number of cycles was then capped at two. The entire dose schedule is represented in Fig. 1A and B.

The MTD was defined as the level in which a DLT occurred in two of three or two of six patients. DLT was defined as any grade 3 or 4 nonhematologic toxicity according to National Cancer Institute criteria version 2.0 excluding nausea, vomiting, or diarrhea in those not receiving adequate premedication. Hematologic toxicity for a DLT included grade IV neutropenia with failure to respond in 10 d to granulocyte colony-stimulating factor, or any duration associated with fever, infection, or thrombocytopenia grade IV, or associated with bleeding or requiring platelet transfusion. Dose escalation to the next level was permitted with a minimum time limit of 4 wk between treatments, with no DLT and evidence of tumor localization (>3% injected radioactivity/kg). The blood count and biochemistry were monitored twice weekly, whereas performance status and toxicity were monitored weekly. Baseline investigations were repeated at day 25 and 53 including tumor response assessment.

Drug formulation and administration. CHT-25 was provided by Novartis and supplied as 10-mg sterile vials lyophilized for reconstitution. ^{131}I was supplied by MDS Nordion or Amersham. Reconstitution of antibody was done during radiolabeling using the *N*-Bromosuccinamide/L-tyrosine technique (22). CHT-25 was administered at a fixed 10-mg dose with a minimum of 8 wk of follow-up, or until toxicity resolved or another anticancer therapy instigated. CHT25 was administered initially at a rate of 6 mg/h, increasing gradually to 24 mg/h over 40 min. 0.9% NaCl was infused concurrently (150 mL/h) with full resuscitation equipment available. An Administration of Radioactive Substances Advisory Committee license was obtained.

Table 1. Patient characteristics

Patient characteristics	No. of patients
No. of patients treated	14*
Sex	
Male	9
Female	5
Age: Median (range) in y	38 (27-70)
Tumor type	
HL	11
ATLL	2
AITCL	1
Performance status	
0	8
1	5
2	1
Ann Arbour stage	
I	0
II	3
III	2
IV	9
B symptoms	
No	5
Yes	9
No of patients receiving "x" prior regimens of chemotherapy	
1	1
2	2
3	4
4	3
5	3
8	1
Previous autologous stem cell transplant	
No	6
Yes	9

Abbreviations: ATLL, adult T cell lymphoma/leukemia; AITCL, angioimmunoblastic T-cell lymphoma.
*One patient was excluded from analysis due to a second malignancy being diagnosed during trial conduct.

Human antimouse antibody response/human antichimeric antibody response. Human antimurine antibody levels were assessed by ELISA prestudy and on days 43 and 57 with RFT5 and the nonspecific isotype matched antibody RDFR2 as positive and negative controls, respectively.

Pharmacokinetic studies. Blood (2.5 mL) was taken in an EDTA blood tube at 1, 3, 6, and 24 h postinfusion then on day 2, 3, 6, and when possible, 9. The samples were stored in an approved radioactive storage area until they had decayed to appropriate levels for analysis. Radioactivity was measured using a Packard Cobra 11th Series Auto γ counter. Monoexponential or biexponential decay curves as appropriate were modeled to the data.

SPECT images were acquired at 4, 24, 48, 72, and 96 h (when possible), for biodistribution, using an ADAC Vertex Plus dual-headed γ camera and reconstructed iteratively (OSEM algorithm; ref. 23) applying scatter and attenuation corrections. Regions of interest were drawn for tumor and organs of interest (heart, lung, liver, spleen, and kidneys) and average radioactivity uptake was then calculated. Olinda/XEM software (24) was used to calculate radiation doses to organs, red marrow (25), and tumor from the radioactivity uptake within the regions of interest, in blood and from the whole body dose-rate data.

Table 2. Toxicity hematological by administered activity and hematological by duration using CTCAE 2.0

A) By administered activity

Administered activity (MBq/m^2)	Haemoglobin nadir (g/dl; median/range)	Neutrophil nadir (×109/liter; median/range)	Platelet nadir (×109/liter; median/range)	Lymphocyte nadir (×109/liter; median/range)
370 (3 treatments)	9.8 (8.4-11.4)	3 (2.7-3.7)	187 (74-195)	0.45 (0.31-0.96)
740 (6 treatments)	9.5 (9-11.5)	4.7 (1.3-11.3)	198 (60-384)	0.38 (0.19-1.55)
1,200 (5 treatments)	8.8 (7.7-10.2)	1.3 (0.9-7.5)	31 (9-83)	0.2 (0.18-1.55)
1,480 (6 treatments)	8.4 (7-10)	0.8 (0.1-7.8)	15 (8-150)	0.2 (0.1-0.58)
>2,220 (4 treatments)	8.5 (6.3-9.5)	1 (0.34-2.7)	13 (8-36)	0.105 (0.06-0.21)

B) Duration of time (in d) at ≥grade 3 or 4 until two readings are recorded for an improved grade or until a second therapy is instigated (which includes a further cycle of CHT25)

Administered activity (MBq/m^2)	370	740	1,200	1,480	2,220	2,960
Grade 3 or 4 platelets	Nil	Nil	Median, 3	Median, 29.5	Median, 33	45 d
median/range (d)	encountered	encountered	Range, 0-60	Range, 0-78	Range, 28-176	(until patient death)
Grade 3 or 4 neutrophils	Nil	Nil	Median, 0	Median, 5.5	Median, 0	38 d
median/range (d)	encountered	encountered	Range, 0-1	Range, 0-30	Range, 0-1	(until patient death)
Grade 4 neutrophils	Nil	Nil	Nil	Median, 2	Median, 0	44 d
median/range (d)	encountered	encountered	encountered	Range, 0-30	Range, 0-1	(until patient death)

Levels of the IL-2 receptor in serum were analyzed by ELISA using the Cellfree Human sIL-2R ELISA kit and a standard methodology.

Assessment of response. Response to treatment was assessed using the updated International Working group Response Criteria (26). An ^{18}F-fluorodeoxyglucose positron emission tomography (PET) scan was done using the hybrid SPECT/PET ADAC Vertex coincidence imaging system (Phillips-ADAC) or GE Discovery LS PET-CT (GE Healthcare) within 2 wk of study entry and then repeated at day 29 and at

Table 3. Drug-related toxicity

Toxicity	Description	Grade 1	Grade 2	Grade 3	Grade 4	Overall
Metabolic	Hypernatraemia	2	0	0	0	2
	Hyponatraemia	1	0	0	0	1
	Elevated urate	1	0	0	0	1
Constitutional	Fatigue	5	0	1	0	6
	Fever	2	0	0	0	2
Hematologic	Anaemia	0	8	3	0	11
	Lymphopenia	0	1	8	0	9
	Leukocytopenia	1	3	3	2	9
	Neutropenia	0	1	2	4	7
	Thrombocytopenia	0	2	3	6	11
Bleeding	Haematuria	2	0	0	0	2
	Vaginal bleeding (menstrual)	0	0	1	0	1
	Petechiae/bruising	1	0	0	0	1
	Epistaxis	0	0	1	0	1
Infection	Febrile neutropenia	0	0	2	1*	3
	Tunneled line infection	0	1	4	0	9
	Upper respiratory tract infection	1	0	1	0	2
	Chest infection	0	1	0	1	2
	Urinary tract infection	0	1	0	0	1
	Oral Candida	2	0	0	0	2
Gastrointestinal	Vomiting	1	0	1	0	2
	Nausea	3	0	0	0	3
	Stomatitis	0	1	0	0	1
Hepatic	Elevated liver enzymes	5	0	0	0	5
Renal	Proteinuria	1	0	0	0	1
Endocrine	Hypothyroidism	0	1	0	0	1
Cardiovascular	Reduction cardiac function	0	1	0	0	1
	Hypotension, day 1	1	0	0	0	1

NOTE: All toxicity seen that was considered possibly, probably, or almost certainly drug related. In addition, it includes all infectious events regardless of presumed causation.
*This patient had grade 5 toxicity and died secondary to PCP and neutropenic sepsis. His death was considered treatment related.

Table 4. Pharmacokinetics

Dose level	Patient no.	A0a(%ID/kg)	A0b(%ID/kg)	T1/2a(h)	T1/2b (h)	50.0%(h)	10.0%(h)
370	1	0.7	3.6	6.1	98.4	73.5	305.5
370	3.1	1.9	6.5	3.2	58.3	37	174
740	3.2		2.7		8	8	28.8
740	4.1	1.8	8.5	0.4	60.4	43.5	186
740	5.1	6	7.1	10.7	422.5	60*	1,025*
740	6	1.3	1.4	0.05	46.3	12.5	120
740	8	3.1	5.5	0.9	57.2	20.5	153
740	9.1		8.2		44.1	44	144
1,200	10.1	3.1	5.9	0.9	86.7	33.5	234
1,200	11.1		15.5		53	53	176
1,200	12.1	7.4	12.7	17.2	113.9	53	300
1,200	12.2		9.6		37.5	37	126
1,480	4.2	4.5	5.8	1.8	135.6	35	338
1,480	5.2	1.9	2.9	16.4	122.7	51	318
1,480	7.1	2.9	7	6.3	76	39	214
1,480	9.2		8		43.9	44	144
1,480	13		5.5		57.8	58	192
1,480	14	2.8	4.5	17	108.1	51	285
2,220	4.3		3.8		123.4	123	410
2,220	7.2		7.9		66.1	66	220
2,220	9.3	1.9	3.4	10.3	58.8	30	156
2,960	7.3		4.6		58.6	58.5	195
Median						44	193.5

Abbreviations: A0a and A0b, amplitudes; T½a, and T½b, half-lives of the first and second components of the biexponential curve; %ID/kg, percentage injected dose per kilogram; 50% and 10%, time taken for modelled activity to fall to 50% or 10% of initial value.
*The long decay shown might have been affected by the small number of blood samples.

day 57. Images were reported from the screen of the camera-dedicated computer by a trained nuclear medicine physician (JB) blinded to patient identity and scan order.

Hematologic toxicity measures were assessed using the blood counts as continuous variables as the outcome measures as well as categorization through the National Cancer Institute Common Toxicity Criteria standard toxicity grading (version 2.0). The clinical characteristics were summarized by simple descriptive summary statistics.

Results

Patient's characteristics. Fifteen patients (Table 1) received one or more treatments; they all had >50% of HL cells or T cells (either malignant or surrounding milieu in HL) positive for CD25. The median number of prior chemotherapy regimens was 4 (range, 1-8); 9 had undergone autologous stem cell transplant. One patient was excluded from analysis because a second biopsy during the study showed the presence of second (B cell) lymphoma that was CD25 negative. Two patients were on long-term steroids (5 and 20 mg prednisolone). Both patients had developed progressive lymphoma on that dose, which remained unchanged throughout the study.

Adverse events. Toxicity is summarized in Tables 2 and 3. There was no significant infusion-related toxicity. The principal toxicity was hematologic and developed progressively as administered activity was escalated up to 2,960 MBq/m^2. The patient treated at this dose had previously received eight lines of therapy and had prolonged myelosuppression after CHT-25. Despite stem cell support, he died at day 36 of neutropenic sepsis and presumed Pneumocystis jiroveci pneumonia. Before treatment, seven patients were lymphopenic (3 grade 2 and 3 grade 3) and counts fell with therapy. On de-escalation to 1,480 MBq/m^2, grade 4 hematologic toxicity occurred (Table 2) with a median platelet nadir of 15 × 10^9/L (range, 8-150) at a median of 41 days (range, 31-56) and neutropenia (median nadir, 0.8 × 10^9/L; range, 0.1-7.8) after a median of 46 days (range, 28-54). A further three patients were treated at 1,200 Mbq/m^2 with acceptable toxicity (Table 2), and this was determined as the MTD.

Blood transfusion was used to maintain the hemoglobin between 12-14 g/L for 4 weeks posttherapy to maximize radiation effect. Granulocyte colony-stimulating factor was given to all patients with grade 4 neutropenia until count recovery. If the neutrophil count remained ≤0.2 × 10^9/L for 2 consecutive days, stem cells were reinfused. Two patients had their stem cells infused, both while suffering from neutropenic sepsis (patient 7 and patient 14 treated at 2,960 MBq/m^2 and 1,480 MBq/m^2, respectively). Six patients required platelet transfusion at or above the MTD of whom five had platelet counts of <10 × 10^9/L. Between a platelet count of 10 to 20 × 10^9/L, administration was at the investigators discretion based on clinical need; below 10 × 10^9/L platelets were given immediately due to risk of spontaneous hemorrhage. All patients receiving platelet transfusions were classified as having grade 4 toxicity. Septic events are included (Table 3) regardless of presumed causation, due to any possible immunomodulatory effect of CHT-25. There was no evidence of viral reactivation.

There were 14 hospital admissions in nine patients. Of these, six were due to infection (four neutropenic sepsis or Hickman line infection with neutropenia), four were associated with progressive disease (one pain, one renal failure secondary to obstruction, two general deterioration), one was for hemoptysis secondary to lymphoma in the lung with a normal platelet

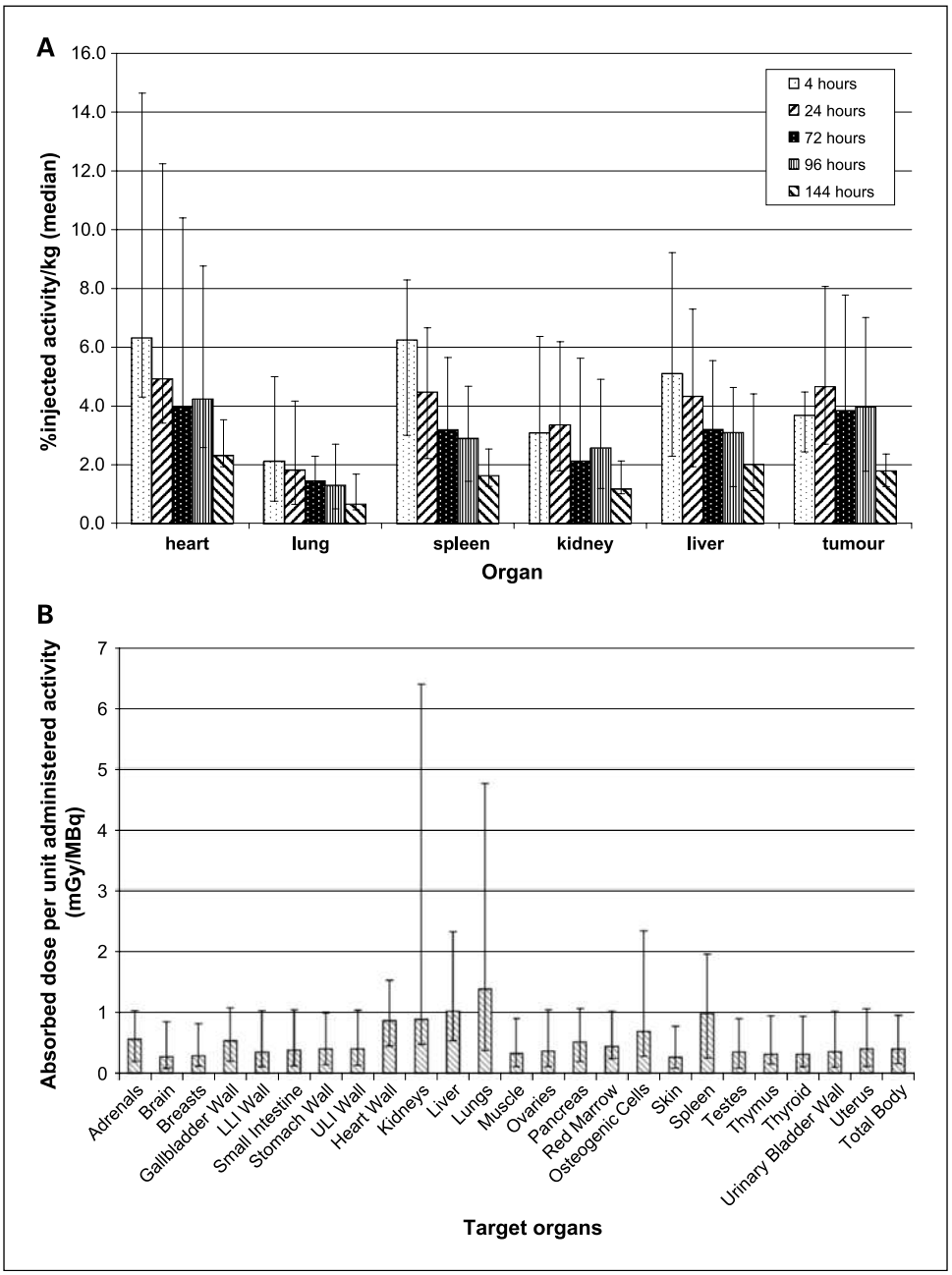

Fig. 2. Distribution of radiation and radiation dosimetry. *A,* mean and ranges of radioactivity uptake by time. *B,* mean and ranges of radiation dose to normal organs including contribution from other organs.

count, one was for 24-h observation after head injury when thrombocytopenic, and two patients for blood transfusion and/or platelet transfusion for practical reasons.

Pharmacokinetics. Table 4 shows the pharmacokinetics for 22 treatments. Of these, 13 can be modeled using a biexponential curve. However, nine patients seem to follow a monoexponential decay: the initial, relatively rapid blood clearance might have been masked by the limited number of data points in the first 6 hours. The 50% and 90% median clearance was calculated to be 44 and 193.5 hours, respectively, consistent with the long half-life of the chimeric antibody.

The median pre treatment soluble IL-2R (sIL2R) was 2,803.3 U/mL (range, 580-66,080 U/mL). This compares with median normal values for the assay of 521 U/mL (range, 269-1,116 U/mL). The level of the pretreatment sIL2R was not found to be a prognostic factor for survival on this small patient sample with patients above 2,803 U/mL having a median survival of 407 days compared with 485 days for those below. (Kaplan-Meier method, Mantel-Cox x^2, 0.17; $P = 0.68$). The molar ratio between the CHT25 antibody and sIL2R levels in plasma at the moment postinjection (plasma volume from standard man) was calculated to be a median of 49 to 1 (with a range from 2:1-260:1). There was no clear relationship in this small sample between the ratio and response.

Immunogenicity. Three patients received three treatments, four had two treatments and seven had one treatment. One patient became HACA response positive (patient 1) at 45 days after the first treatment. There was rapid blood clearance of CHT-25 in patient 3 without positive HACA and this was not explained. Patient 9 was nonspecifically positive in the HACA

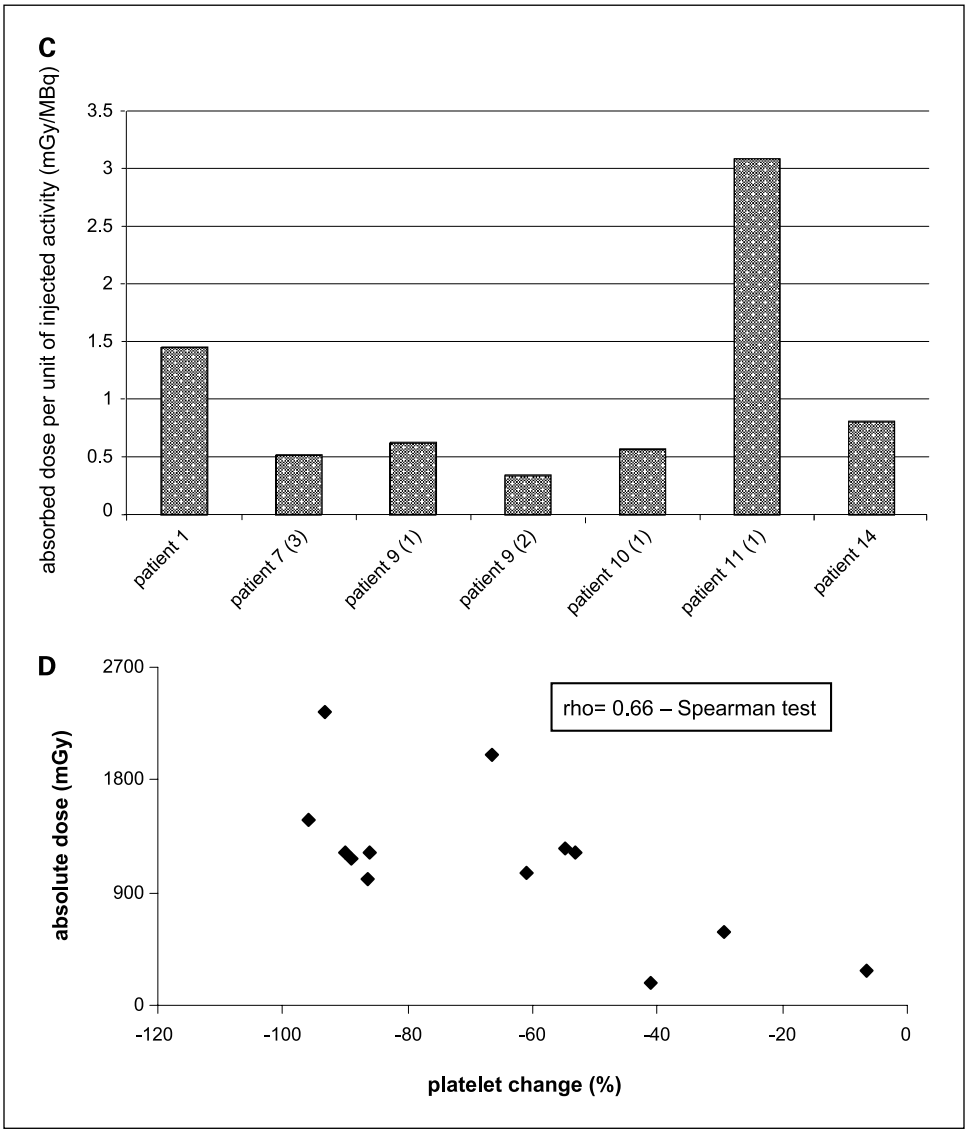

Fig. 2 *Continued. C* radiation dose to tumor excluding contribution from normal organs. *D,* correlation between % change from baseline of platelet count nadir and absorbed red marrow dose in first cycle.

assay to both irrelevant control antibody and PBS at baseline; values fell with therapy suggesting nonspecific binding. One patient was screened for entry to the trial after an allograft but was ineligible due to a positive HACA possibly representing an immune antitumor response.

Distribution of radioactivity and dosimetry. Data were collected on 11 patients. Figure 2A shows median values and ranges of the percent of injected radioactivity per kilogram in normal organs and tumor with wide interpatient variation. While radioactivity declined from 4 hours in normal tissues, it increased up to 24 hours in the tumor with retention to 96 hours Comparing tumor to blood ratio 4 hours after administration with those after 96 hours, there was an increase in all seven cases with sufficient data for this analysis by a median of 2.2-fold (range, 1.3:1-4.0:1; $P = 0.005$ by the Wilcoxon Signed Ranks test). Figure 2B shows the absorbed radiation dose in normal tissues (Olinda/XEM software; ref. 24). Figure 2C shows the absorbed radiation dose in tumors of individual patients (Olinda/XEM). Tumor values are not comparable with those for normal tissues (Fig. 2B) because this software does not include the contribution to radiation dose from adjacent tissues as it does for normal tissues. Figure 2D shows the relationship between the absorbed bone marrow dose per unit of injected activity and bone marrow toxicity (Spearman rank value r = -0.66). In addition, a relationship was seen between absorbed tumor dose and response in seven patients. Nonresponders had tumor doses of ≤1,325 mGy and responders had doses of ≥1,990 mGy with no crossover. (Mann-Whitney $P = 0.057$).

Clinical responses. Objective responses were observed in an administered activity-dependent fashion (Table 5). No responses were seen at 370 MBq/m^2 and one at 740 MBq/m^2. Six of nine patients treated at a single administration of 1,200 MBq/m^2 or higher had a response [three complete responses (CR; two HL, and one AITCL) and three partial response (PR; all HL); Fig. 3]. The two patients with adult T-cell leukemia/lymphoma had ≤740 MBq/m^2 and one had a CR.

Four patients (patient 10, 11, 12, and 14) considered ineligible for allogeneic or autologous transplant because of chemoresistant disease were treated. Three responded; two

proceeded with transplant, one entered CR comorbidity prevented transplant.

Survival. The median overall survival was 453 days (range, 44-925). Two patients remain alive at the point of data analysis. The progression-free survival of patients who had a response or stable disease was a median of 179 days (range 72-925+). The two patients transplanted post–CHT-25 were excluded from progression-free survival analysis.

Discussion

There is limited experience with chimeric or human antibodies in the RIT of HL or T-cell lymphomas. A phase I study of a chimeric antibody demonstrating favorable findings at the MTD is reported. It is relevant when considering toxicity and response that the patients had poor prognostic features including multiple lines of prior myelosuppressive therapy. All tumors were positive for CD25 on immunohistochemistry; with a median duration between CD25 staining and treatment of 384 days (range, 39-2,722 days), dedifferentiation and a loss of CD25 status cannot be excluded. Good localization of CHT25 by SPECT imaging suggests that dedifferentiation was not an issue.

Dose-limiting toxicity was myelosuppression, which was readily managed at the MTD. The only treatment-related death occurred in a patient who received more than twice this dose. The rate of non-neutropenic infection was relatively high; this may have been related to the use of long-term central venous access devices. Modulation of CD25-positive immune cells by therapy and other immune defects consequent on previous therapies cannot be excluded. There was no evidence of viral reactivation, but given the associated lymphopenia, appropriate microbial prophylaxis for lymphopenic patients may be warranted. Repeated therapy was feasible after recovery from hematologic toxicity and further chemotherapy was not precluded in patients whose tumor did not respond.

Response assessed using ^{18}F-fluorodeoxyglucose PET and CT (26–28) was encouraging. Of nine patients treated at or above the MTD, the overall response rate was 67% (three CR and three PR). Two further patients had stable disease, whereas one had progression. The two patients on stable doses of prednisolone responded to CHT25 having previously progressed while taking this steroid dose. The study was not designed to assess progression-free survival or overall survival, and the figures presented in the results section were derived from routine clinical follow-up. It is evident that long-term responses and survival can be achieved.

Pharmacokinetics and dosimetry showed that the chimeric antibody delivered radiation to normal tissues over a more prolonged time than previously reported in a murine CD25 antibody, although with wide variability (^{90}Y-antiTac; ref. 29). To exploit this, ^{131}I with its physical half-life of 8 days was chosen as the therapeutic radionuclide. One advantage of using ^{131}I is the mean path length (0.4 mm) of the radionuclide, which allows for the eradication of adjacent antigen-negative tumor cells. The longer path length of ^{90}Y means a greater proportion of the dose is deposited outside small tumor masses and its shorter half-life means that the prolonged retention of antibody in tumor cannot be exploited. However, there is potential for investigation of other β-emitting radionuclides.

Serum levels of sIL2R were elevated in all patients but antibody was calculated to remain in excess at the time of administration. Because satisfactory tumor targeting was shown and tumor response rate was high, it is concluded that the antibody/antigen ratios were satisfactory for effective therapy. However, it remains possible that further improvements in antibody delivery could be achieved by reducing sIL2R levels before therapy.

Previous studies have shown that an elevated sIL2R level is an adverse prognostic factor in HL (30) and decreases in responding disease. This was not confirmed in the present study but the sample size was small.

There was a correlation between red marrow–absorbed dose and marrow toxicity, although variation in the extent of prior bone marrow damage is a separate parameter and was not considered. Flt-3 levels have been reported as a means of addressing this issue (31) and would be worthy of inclusion in future trials. A clear dose response relationship was not seen

Table 5. Patient response data with PET and CT

Patient number	Administered activity (MBq/m^2)	PET	CT (WHO criteria)	Combined (Cheson criteria)
1	370	Not done	Not evaluable	Clinical PD
2	370	Not done	PD	PD (CT alone)
3	370	PR	SD	SD
4	740, 1,480, 2,220	CR	PR	CR
5	740, 1,480	CR	CR	CR
6	740	PD	SD	PD
7	1,480,2,220, 2,960	PR	PR	PR
8	740	Not done	Not done	Clinical PD
9	740, 1,480, 2,220	SD	SD	SD
10	1,200	PR	SD	SD
11	1,200, 1,200	PR	PR	PR
12	1,200, 1,200	CR	SD	CR
13	1,480	PD	PD	PD
14	1,480	PR	PR	PR

NOTE: Table of responses: best response with first scan at 4 wk and confirmed at 8 wk.
Abbreviations: PD, progressive disease; SD, stable disease.

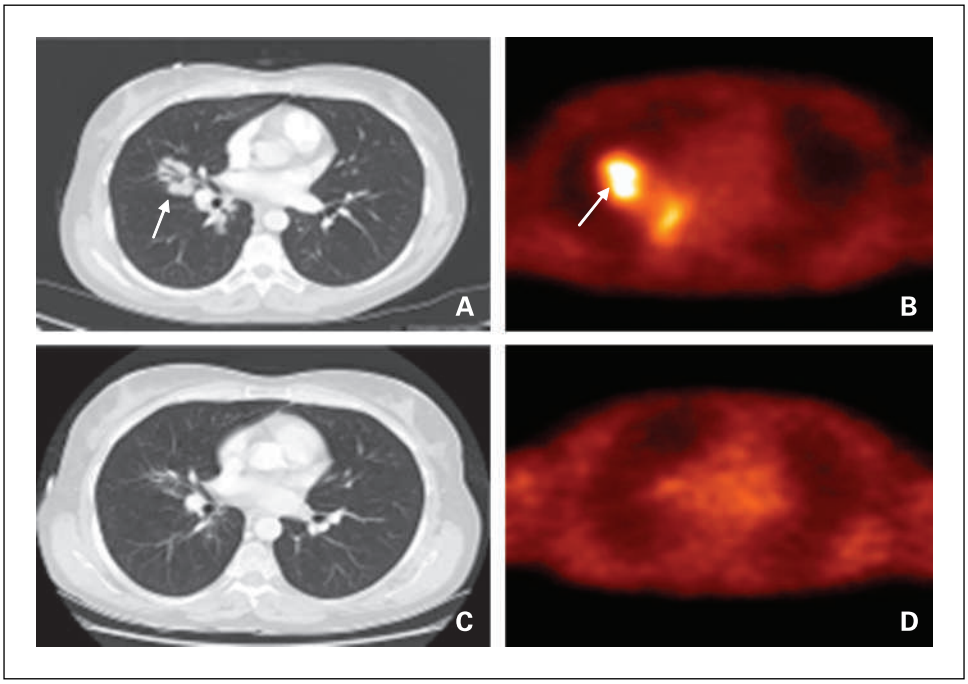

Fig. 3. Patient response data with PET and CT. *A,* CT and PET images demonstrating PR with FDG-PET and CT. *A* and *B,* pretreatment showing nodal disease (*arrow*). *C* and *D,* 4 wk posttreatment.

in this small study; however, tumor responses were only recorded in patients with absorbed doses of ≥1,990 mGy. One of the objectives of the planned phase II study will be to determine whether this is a consistent finding so that pretreatment dosimetry (including blood pharmacokinetics) could be used to select appropriate patients for therapy.

Although the radiation dose seems important for tumor response, an additional cytotoxic effect may be produced by the antibody. Recent work has considered a possible anticancer effect from the depletion of regulatory T cells (32) or through other immune effects. Currently evidence of a direct anticancer-immune mediated antibody effect is inconclusive. Of note, unlabeled murine antibody without a radioconjugate showed only limited benefit with responses of a short-lived duration (10). For this reason, CHT25 was not trialed in its unlabeled form as an anticancer agent. There is no preclinical, anticancer data for CHT25 due to a lack of a suitable animal model; although rhesus monkeys share the same IL-2R epitope as humans, they are not suitable to be used in therapy studies.

Previously phase I/II studies in HL using RIT have combined ^{131}I with polyclonal anti-ferritin or anti-CD30–directed antibodies (33, 34). The overall response rates have ranged from 36% to 49% and the major side effect, as observed here, was hematologic toxicity, most notably thrombocytopenia. ^{131}I-Rituximab has shown good effect in 91 patients with relapsed or refractory NHL achieving an overall response rate of 76% with 53% attaining either a CR or a CR, unconfirmed (Cru). These results are similar to the two licensed murine antibodies; the degree of myelosuppression was similar degree even with the longer circulating time of the chimeric antibody (35).

CHT25 has a potential application in preparation for autologous and allogeneic transplantation, giving the possibility of long-term disease control to a proportion of patients with relapsed or refractory disease. Demonstrable chemosensitivity and maximal reduction in disease burden before transplantation are critical predictors of transplant outcome in HL (2, 36). In this study, we induced PET responses and transplanted patients previously considered inappropriate for this treatment. This justifies further investigation of CHT25 either to render refractory HL patients eligible for transplant or to improve transplant outcome when combining it with standard conditioning regimens. It may also have a place in the early treatment of poor prognosis patients and other CD25-positive lymphomas as it is well known that a range of hematologic malignancies express CD25 (37–41). In our study, we observed a CR in a patient with a T-cell lymphoma, justifying further studies to look at this patient group.

In summary, the CHT25 study shows that RIT with a chimeric antibody can be safely administered at a dose of 1,200 MBq/m^2, giving objective responses in a high proportion of patients with lymphomas expressing CD25. Pharmacokinetic and pharmacodynamic parameters relevant to producing this effect have been shown. CHT25 seems to have potential as a new treatment in this patient group and is shortly to proceed to further evaluation of efficacy in phase II studies.

Disclosure of Potential Conflicts of Interest

CHT25 is described in U.S. Patent 6,383,487; held by P. Amlot, A. Akbar, G. Heinrich, and S. Cammisuli. P. Amlot is coauthor of U.S. Patent 6,383,487. The other authors declare they have no conflict of interest.

Acknowledgments

We thank Peter Isaacson for assessment of CD25 staining, Andrew Hilson for image reading and administrative support, Ama Rohatiner, Jamie Cavenagh, Ben Mead, Peter Johnson, Kim Orchard, Alan Horwich, Anthony Goldstone, and the hematology department at the Royal Free Hospital for support of the study.

References

1. Martin A, Fernandez-Jimenez MC, Caballero MD, et al. Long-term follow-up in patients treated with Mini-BEAM as salvage therapy for relapsed or refractory Hodgkin's disease. Br J Haematol 2001;113:161–71.
2. Schmitz N, Pfistner B, Sextro M, et al. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. Lancet 2002;359:2065–71.
3. Waldmann TA, Goldman CK, Robb RJ, et al. Expression of interleukin 2 receptors on activated human B cells. J Exp Med 1984;160:1450–66.
4. Waldmann TA. The structure, function, and expression of interleukin-2 receptors on normal and malignant lymphocytes. Science 1986;232:727–32.
5. Sheibani K, Winberg CD, van d V, et al. Distribution of lymphocytes with interleukin-2 receptors (TAC antigens) in reactive lymphoproliferative processes, Hodgkin's disease, and non-Hodgkin's lymphomas. An immunohistologic study of 300 cases. Am J Pathol 1987;127:27–37.
6. Agnarsson BA, Kadin ME. The immunophenotype of Reed-Sternberg cells. A study of 50 cases of Hodgkin's disease using fixed frozen tissues. Cancer 1989;63:2083–7.
7. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235–42.
8. Herold M, Haas A, Srock S, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. J Clin Oncol 2007;25:1986–92.
9. Ansell SM, Horwitz SM, Engert A, et al. Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. J Clin Oncol 2007;25:2764–9.
10. Waldmann TA, Goldman CK, Bongiovanni KF, et al. Therapy of patients with human T-cell lymphotrophic virus I-induced adult T-cell leukemia with anti-Tac, a monoclonal antibody to the receptor for interleukin-2. Blood 1988;72:1805–16.
11. Barth S, Huhn M, Matthey B, et al. Ki-4(scFv)-ETA', a new recombinant anti-CD30 immunotoxin with highly specific cytotoxic activity against disseminated Hodgkin tumors in SCID mice. Blood 2000;95:3909–14.
12. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. J Clin Oncol 2000;18:1622–36.
13. Waldmann TA, White JD, Carrasquillo JA, et al. Radioimmunotherapy of interleukin-2R α-expressing adult T-cell leukemia with Yttrium-90-labeled anti-Tac. Blood 1995;86:4063–75.
14. Waldmann TA. Anti-IL-2 receptor monoclonal antibody (anti-Tac) treatment of T-cell lymphoma. Important Adv Oncol 1994:131–41.
15. Hakimi J, Chizzonite R, Luke DR, et al. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. J Immunol 1991;147:1352–9.
16. Novartis Pharmaceutical Corporation. Simulect (CHT-25). 2003.
17. Amlot PL, Rawlings E, Fernando ON, et al. Prolonged action of a chimeric interleukin-2 receptor (CD25) monoclonal antibody used in cadaveric renal transplantation. Transplantation 1995;60:748–56.
18. Nashan B, Moore R, Amlot P, et al. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. CHIB 201 International Study Group. Lancet 1997;350:1193–8.
19. Kaminski MS, Zasadny KR, Francis IR, et al. Radioimmunotherapy of B-cell lymphoma with [^{131}I]anti-B1 (anti-CD20) antibody. N Engl J Med 1993;329:459–65.
20. Kaminski MS, Estes J, Zasadny KR, et al. Radioimmunotherapy with iodine (131)I tositumomab for relapsed or refractory B-cell non-Hodgkin lymphoma: updated results and long-term follow-up of the University of Michigan experience. Blood 2000;96:1259–66.
21. Witzig TE, Molina A, Gordon LI, et al. Long-term responses in patients with recurring or refractory B-cell non-Hodgkin lymphoma treated with yttrium 90 ibritumomab tiuxetan. Cancer 2007;109:1804–10.
22. Adam T. Radioiodination for therapy. Ann Clin Biochem 1989;26:244–5.
23. Green AJ, Dewhurst SE, Begent RH, et al. Accurate quantification of 131I distribution by γ camera imaging. Eur J Nucl Med 1990;16:361–5.
24. 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.
25. Wessels BW, Bolch WE, Bouchet LG, et al. Bone marrow dosimetry using blood-based models for radiolabeled antibody therapy: a multiinstitutional comparison. J Nucl Med 2004;45:1725–33.
26. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007;25:579–86.
27. Shankar LK, Hoffman JM, Bacharach S, et al. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med 2006;47:1059–66.
28. Jabbour E, Hosing C, Ayers G, et al. Pretransplant positive positron emission tomography/gallium scans predict poor outcome in patients with recurrent/refractory Hodgkin lymphoma. Cancer 2007;109:2481–9.
29. Carrasquillo JA, White JD, Paik CH, et al. Similarities and differences in 111In- and 90Y-labeled 1B4M-DTPA antiTac monoclonal antibody distribution. J Nucl Med 1999;40:268–76.
30. Gause A, Roschansky V, Tschiersch A, et al. Low serum interleukin-2 receptor levels correlate with a good prognosis in patients with Hodgkin's lymphoma. Ann Oncol 1991;2 Suppl 2:43–7.
31. Siegel JA, Yeldell D, Goldenberg DM, et al. Red marrow radiation dose adjustment using plasma FLT3-L cytokine levels: improved correlations between hematologic toxicity and bone marrow dose for radioimmunotherapy patients. J Nucl Med 2003;44:67–76.
32. Koenecke C, Ukena SN, Ganser A, et al. Regulatory T cells as therapeutic target in Hodgkin's lymphoma. Expert Opin Ther Targets 2008;12:769–82.
33. Schnell R, Dietlein M, Staak JO, et al. Treatment of refractory Hodgkin's lymphoma patients with an iodine-131-labeled murine anti-CD30 monoclonal antibody. J Clin Oncol 2005;23:4669–78.
34. Lenhard RE, Jr., Order SE, Spunberg JJ, et al. Isotopic immunoglobulin: a new systemic therapy for advanced Hodgkin's disease. J Clin Oncol 1985;3:1296–300.
35. Leahy MF, Seymour JF, Hicks RJ, et al. Multicenter phase II clinical study of iodine-131-rituximab radioimmunotherapy in relapsed or refractory indolent non-Hodgkin's lymphoma. J Clin Oncol 2006;24:4418–25.
36. Majhail NS, Weisdorf DJ, Defor TE, et al. Long-term results of autologous stem cell transplantation for primary refractory or relapsed Hodgkin's lymphoma. Biol Blood Marrow Transplant 2006;12:1065–72.
37. Kodaka T, Uchiyama T, Ishikawa T, et al. Interleukin-2 receptor β-chain (p70-75) expressed on leukemic cells from adult T cell leukemia patients. Jpn J Cancer Res 1990;81:902–8.
38. Strauchen JA, Breakstone BA. IL-2 receptor expression in human lymphoid lesions. Immunohistochemical study of 166 cases. Am J Pathol 1987;126:506–12.
39. Chott A, Augustin I, Wrba F, et al. Peripheral T-cell lymphomas: a clinicopathologic study of 75 cases. Hum Pathol 1990;21:1117–25.
40. Doi S, Nasu K, Arita Y, et al. Immunohistochemical analysis of peripheral T-cell lymphoma in Japanese patients. Am J Clin Pathol 1989;91:152–8.
41. Ralfkiaer E, Wantzin GL, Stein H, et al. T-cell growth factor receptor (Tac-antigen) expression in cutaneous lymphoid infiltrates. J Am Acad Dermatol 1986;15:628–37.

Clinical Cancer Research

A Phase I Clinical Trial of CHT-25 a ^{131}I-Labeled Chimeric Anti-CD25 Antibody Showing Efficacy in Patients with Refractory Lymphoma

Gairin Dancey, John Violet, Alessandra Malaroda, et al.

Clin Cancer Res 2009;15:7701-7710.