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Clinical Trials With an Anti-CD25 Ricin A-Chain Experimental and Immunotoxin (RFT5-SMPT-dgA) in Hodgkin's Lymphoma

R. SCHNELL[a], E. VITETTA[b], J. SCHINDLER[b], S. BARTH[a], U. WINKLER[a], P. BORCHMANN[a], M. L. HANSMANN[c], V. DIEHL[a], V. GHETIE[b] and A. ENGERT[a,*]

[a]*Klinik I fuer Innere Medizin, Universitaet zu Koeln, 50924 Koeln, Germany;* [b]*Cancer Immunobiology Center and Department of Microbiology, The University of Texas, Southwestern Medical Center, Dallas, Texas 75235, U.S.A. and* [c]*Senkenbergisches Zentrum der Pathologie, Johann Wolfgang Goethe-Universitaet Frankfurt a.M., 60596 Frankfurt a.M., Germany*

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Immunotoxins (ITs) consisting of a cell-binding component and a potent toxin were developed as a new class of biological anti-tumor agents to improve adjuvant therapy. Hodgkin's lymphoma (HL) has been demonstrated to be an excellent target for ITs because high concentrations of lymphocyte activation markers such as CD25 and CD30 are expressed on Hodgkin and Reed-Sternberg (H-RS). Several ITs against these antigens have shown potent antitumor effects against H-RS cells *in vitro* and in different HL animal models. On the basis of its superiority in preclinical models, the anti-CD25 IT RFT5-SMPT-dgA was subsequently evaluated in a phase I study in patients with refractory Hodgkin's lymphoma. The IT was constructed by linking the monoclonal antibody (Moab) RFT5 via a sterically hindered disulfide linker (SMPT) to deglycosylated ricin A-chain (dgA). All 15 patients enrolled in this trial were heavily pretreated with a mean of five different prior therapies. The IT was administered intravenously over four hours on days 1-3-5-7 for total doses per cycle of 5, 10, 15, or 20 mg/m^2. Side effects were reversible and related to the vascular leak syndrome (VLS), i.e. decrease in serum albumin, edema, weight gain, hypotension, tachycardia, myalgia, and weakness. In all three patients receiving 20 mg/m^2 NCI toxicity grade III was observed. Thus, 15 mg/m^2 is the maximal tolerated dose (MTD) of RFT5-SMPT-dgA. 50% of the patients developed human anti-ricin A-chain antibodies (HARA) and/or human anti-mouse antibodies (HAMA). Clinical results included two partial remissions (PR), one minor response (MR), three stable disease (SD) and nine progressive disease (PD). In an extension of the phase I trial, five additional patients have been treated at the MTD.

Keywords: Immunotoxin, RFT5-SMPT-dgA, CD25, Hodgkin's lymphoma

*Corresponding author. Fax: +49 (0) 221 / 478-63 83.

INTRODUCTION

Polychemotherapy and extended field radiotherapy have improved the remission rates of Hodgkin's lymphoma (HL) from less than 5% in 1963 to about 80% at present.[1] However, 30–50% of patients with advanced stages die due to relapsed disease.[2] Data from other malignancies including colorectal cancer,[3] myeloid leukemia,[4] or non-Hodgkin's lymphoma (NHL)[5] indicate that small numbers of residual tumor cells remaining after first-line treatment are the source of relapses. Thus, the elimination of residual tumor cells after first-line treatment might improve the outcome in malignant diseases including HL.

There are several approaches to the elimination of residual tumor cells including T-cell stimulation, vaccination or selective destruction using Moabs or Moab-based constructs.[6–8] Since native Moabs have been ineffective against H-RS cells thus far,[9] constructs consisting of a specific cell-binding moiety and a potent toxin have been developed. These immunotoxins (ITs) are ideally suited to kill residual tumor cells in HL for several reasons: 1. H-RS cells express large numbers of surface antigens such as CD15,[10] IRac,[11] CD30,[12] CD25,[13,14] CD40,[15] and CD80 (B7-1),[16] which are present only on a minority of normal human cells. 2. The number of H-RS cells that need to be killed is relatively small. 3. Hodgkin's tumors are well vascularized, suggesting good access of the IT to the target cells. 4. The mechanism of cell killing of ITs is completely different from that of conventional agents. 5. ITs are capable of killing dormant non-dividing cells.

There are two lymphoid activation markers, CD25 and CD30, which have attracted great interest as targets for ITs in HL. Our group has evaluated most Moabs available against CD25 and CD30 in terms of their ability to form ricin A-chain ITs for possible clinical use in HL. In this paper, we will focus on ITs against CD25.

CD25 is the α-chain of the IL-2 receptor which is composed of three different membrane components termed α-, β-, and γ-chain.[17] Combinations of these chains result in different forms of the IL-2 receptor with distinct binding affinities for IL-2. CD25 is a 55 kd glycoprotein which is not expressed on resting lymphocytes and stem cells but which is efficiently induced upon T-cell activation. CD25 binds IL-2 with low affinity without signal transduction activity. IL-2 receptors have been detected in high copy numbers in hematopoietic malignancies and autoimmune disorders including HL.[18,19]

Preclinical Evaluation of Ricin A-Chain ITs Against CD25

Twenty-three different Moabs against CD25 were tested in an indirect assay[20] for their potential use as ITs against Hodgkin-derived cell lines such as L428 and L540.[21] The five most potent Moabs were subsequently coupled to deglycosylated ricin A-chain via SMPT, and the cytotoxicity of the constructed ITs was determined in a standard 3H-leucine uptake assay.[22] The most potent IT, RFT5-SMPT-dgA, inhibited the protein synthesis of L540 cells by 50% at a concentration (IC_{50}) of 7×10^{-12} M, which is identical to that of native ricin under the same experimental conditions.[23] RFT5 itself showed no major crossreactivity with any tissues other than lymphoid, where a few large cells in tonsils and lymph nodes were stained (Table I).[23]

The anti-tumor activity was evaluated in triple-beige nude mice with subcutaneously growing solid Hodgkin's tumors of 60–80 mm^3. A single i.v. application of 8 μg (in terms of A-chain) RFT5-SMPT-dgA[23] induced permanent complete remissions in 78% of the animals. In contrast, 100% of the control animals showed progressive tumor growth. The tumor size at the time of IT application significantly influenced the response rates: only 37.5% complete remissions occurred in animals with larger tumors (10 mm diameter), whereas 100% of the mice with smaller tumors (3 mm) achieved complete remissions. Treatment of disseminated growing Hodgkin's lymphoma was performed in SCID mice:[24] After administration of 8 μg RFT5-SMPT-dgA (in terms of A-chain) intraperitoneally one day after i.v. inoculation of 1×10^7 L540Cy Hodgkin-derived cells, complete remissions were observed in 95% (22/23). In contrast, 92% (34/37) of untreated SCID mice showed signs of progressive

TABLE I Normal tissue staining patterns of CD25 antibodies

Tissue	anti-CD25 antibody			
	B-B10	B-F2	RFT5γ2a	RFT5γ1
Adrenal	–	–	–	–
Brain (cortex)	–	–	–	–
Brainstem	–	–	–	–
Breast	–	–	–	–
Cerebellum	–	–	–	–
Cervix	–	++	–	–
Colon	–	–	–	–
Gall bladder	–	–	–	–
Heart	–	–	–	–
Ileum	–	++	–	–
Kidney	–	+++	–	–
Liver	–	–	–	–
Lung	–	–	–	–
Lymph node	–	–	–	–
Mucosa (nasal)	–	–	–	–
Oesophagus	–	–	–	–
Ovary	–	–	–	–
Pancreas	–	–	–	–
Parathyroid	–	–	–	–
Spleen	–	–	–	–
Stomach (antrum)	–	+	–	–
Stomach (body)	–	+	–	–
Testis	–	–	–	–
Thyroid	–	–	–	–
Thyroid (AI)	–	–	–	–
Thyroid (Hashimoto's)	–	–	–	–
Tonsils	–*	–*	–*	–*
Uterus	–	–	–	–
Vagina	–	–	–	–
Hodgkin's disease	+++	+++	+++	+++

* Rare cells within lymphoid tissue stain positively.

tumor growth. The mean survival time (MST) of SCID mice treated with RFT5-SMPT-dgA one day after tumor challenge was >180 days as compared to 36 days in PBS and 48 days in Moab-treated controls (Fig. 1).

Clinical Phase I Trial with the CD25 Ricin A-chain IT RFT5-SMPT-dgA

RFT5-SMPT-dgA was the most potent IT against human Hodgkin's lymphoma *in vitro* and in animal models combining strong staining of H-RS cells and little crossreactivity.[23,24] Thus, RFT5-SMPT-dgA was selected for an FDA-approved phase I trial in patients with relapsed refractory HL.[25] The patient formulation and characteristics of the IT are summarized in Table II. The study design was in accordance with the Declaration of Helsinki. The trial was approved by the Ethics Committees of the University of Cologne and the University of Texas, Southwestern Medical Center, and performed under Food and Drug Administration Investigational New Drug Application (IND No 4989). Before treatment, all patients gave written informed consent. The IT was given intravenously (i.v.) in 100 ml isotonic saline over 4 hours on days 1-3-5-7. Cohorts with a minimum of three patients were treated with escalating doses of 5, 10, 15, and 20 mg/m^2. If one patient experienced grade III toxicity three additional patients were enrolled at this dose level. If three grade III toxicities occurred at one dose level, then the previous dose level was regarded

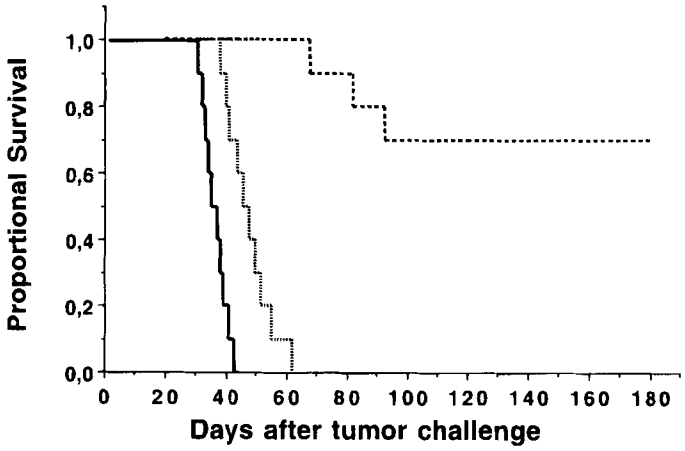

FIGURE 1 Mean survival time of SCID mice bearing disseminated Hodgkin-derived L540Cy tumors. One day after tumor challenge with 1×10^7 L540Cy cells groups of 10 mice were treated with PBS (———), 40μg of the native antibody RFT5 (·····) or 8μg (in terms of A-chain) of the IT RFT5-SMPT-dgA (- - -).

as the MTD. If two patients at one dose level experienced a grade III toxicity and one patient at the next dose level a grade IV toxicity, then the MTD was the dose at which the grade III toxicities occurred. If one patient experienced a grade III toxicity and another patient experienced a grade IV toxicity, then the previous dose level was defined as the MTD.

A total of 15 patients with refractory or relapsed (≥2 relapses) progressive HL were included in the phase I trial. Five additional patients treated at the MTD are included in this report.

Patient Characteristics and Tumor Pathology

The demographic data of the 15 patients treated in the phase I trial and the additional five patients treated at MTD are listed in Table III. Twelve patients were male and eight were female. The median age was 29 (range 19 to 38). Histopathology at first presentation was nodular sclerosis in most cases (14), followed by mixed cellularity (4), lymphocyte depletion (1), and lymphocyte predominance (1). Of 20 patients, eight suffered from primary progressive disease. Most

TABLE II Patient formulation and characteristics of RFT5-SMPT-dgA

Sterility	Sterile
Endotoxin (Limulus amebocyte lysate assay)	0.3 Endotoxin units/ml
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (% as M_r 180.000 band)	70%
Binding to thiopropyl-Sepharose 6 B (free-sulfhydryl groups)	none
A-chain activity relative to native dgA	100%
Phytohemagglutinin activity for human peripheral blood mononuclear cells	2.8×10^{-10} M
Antibody binding activity relative to native antibody	100%
Antibody subclass	IgG1
Target antigen	CD25
Crossreactivity	Activated lymphocytes
IC_{50} against L540	7×10^{-12} M
LD_{50}	9 μg/g mouse

Abbreviations: IC50, inhibitory concentration of 50%; LD50, lethal dose of 50%; dgA, deglycosylated ricin A-chain.

TABLE III Characteristics of patients treated with RFT5-SMPT-dgA

ID	dose (mg/m2)	Age	Gender	Histology	Primary resistant	Prior therapies	ABMT/ PSCT	Karnofsky index	Stage
1	5	23	f	LP	N	5	N	50	IVB
2	5	27	m	NS	N	5	Y	70	IVA
3	5	34	m	NS	N	8	Y	70	IVB
4	10	33	m	MC	N	5	Y	50	IVB
5	10	31	f	NS	N	4	Y	90	IVA
6	10	28	m	NS	Y	6	N	60	IVB
7	15	32	f	NS	N	5	Y	80	IVA
8	15	19	m	MC	Y	2	N	80	IVA
9	15	34	m	NS	N	6	Y	70	IVA
10	15	31	m	MC	Y	5	Y	90	IVA
11	15	20	f	MC	N	3	N	90	IIA
12	15	33	m	NS	N	3	Y	80	IVB
13	20	28	m	NS	Y	3	N	60	IVB
14	20	29	f	NS	Y	6	N	90	IVA
15	20	33	m	NS	N	3	N	90	IIA
16	15	37	m	LP	Y	5	N	70	IIA
17	15	19	m	NS	Y	6	Y	70	IVB
18	15	38	f	NS	N	4	N	80	IIIA
19	15	23	f	NS	Y	3	Y	80	IVA
20	15	36	f	NS	N	3	N	80	IVB

Abbreviations: f, female; m, male; NS, nodular sclerosis; MC, mixed cellularity; LP, lymphocyte predominance; LD, lymphocyte depletion; N, no; Y, yes; ABMT, autologous bone marrow transplantation; PSCT, peripheral stem cell transplantation; ID, identification number.

patients had been heavily pretreated with an average of 4.5 different therapies (range 2–8) including high-dose chemotherapy (HDCT) and autologous bone marrow transplantation in 50%. All but one patient (no. 8) had also received extensive radiotherapy. At study entry, the median performance status as measured by the Karnofsky index was 70 (range: 50–90). Most patients presented with advanced disease (stage IV: 16/20) and 8 had B symptoms. Six patients were on steroids to control excessive fever or sweating.

The evaluation of CD25 expression on H-RS cells was hindered by the difficulty in obtaining sufficient biopsy material (Table IV). A total of nine lymph node biopsies were performed of which eight contained more than 30% of H-RS cells expressing the CD25 antigen. In two additional patients, material from the lung (no. 9) and liver (no. 12) contained >30% $CD25^+$ H-RS cells. CD25 expression was less pronounced than CD30 except in patient no. 10, who demonstrated positive staining in 40% vs 10%. In general, the percentage of CD25-positive small lymphoid cells in the tissues analyzed was less than 1% (data not shown).

Toxicity

None of the six patients treated at the first two dose levels (5 mg/m^2 and 10 mg/m^2) experienced toxicity higher than grade II. Since patient no. 7 experienced grade III toxicity (myalgia, CK_{max} 200 U/ml) at the 15 mg/m^2 dose level, three further patients had to be treated at that dosage (total of six). Only one of the additional patients experienced grade III toxicity (dyspnea) (patient no. 12), thus dose escalation was possible. Three patients were treated at 20 mg/m^2. Patient no. 15 received only three of the four planned IT infusions due to a grade IV myalgia on day 6 with a CK max of 1,500 U/ml. This reaction was rapidly reversible after the cessation of IT therapy and treatment with 24 mg/d dexamethasone for four days. At least one grade III toxicity was observed in all 3 patients at this dose level. Two patients experienced VLS grade III with weight gain > 15 lb; two patients had grade III nausea/vomiting requiring antiemetics, and tachycardia (>140 bpm at rest) occurred in two patients, making the administration and beta-blocker necessary. Thus, the MTD of RFT5-SMPT-dgA was

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