Clinical evaluation of ricin A-chain immunotoxins in patients with Hodgkin’s lymphoma

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Background: Immunotoxins (ITs) consist of cell binding ligands coupled to toxins or their subunits. Hodgkin’s lymphoma (HL) is an excellent target for ITs since lymphocyte activation markers such as CD25 and CD30 are expressed in large numbers. The ITs RFT5.dgA (anti CD25) and Ki-4.dgA (anti CD30) were constructed by linking the monoclonal antibodies RFT5 and Ki-4 to deglycosylated ricin A-chain (dgA). Both ITs showed potent specific activity against HL cells in vitro and in vivo in animal models, and were subsequently evaluated in phase I/II clinical trials in humans.

Patients and methods: In two separate trials, the ITs were administered i.v. four times every other day over 4 h. The objectives of the phase I trials included the determination of the maximum tolerated dose (MTD), dose-limiting toxicities (DLTs), pharmacokinetics, antitumor activity and immune response against the IT.

Results: Twenty-seven patients with refractory HL were included in the phase I/II study of RFT5.dgA and 17 patients were included in the phase I study of Ki-4.dgA. The MTD of RFT5.dgA was 15 mg/m², whereas that of Ki-4.dgA was 5 mg/m². DLTs were related to vascular leak syndrome, consisting of edema, tachycardia, dyspnea, weakness and myalgia. Measurement of serum levels of RFT5.dgA demonstrated a Cmax of 0.2–9.7 µg/ml with a half-life (t½) varying from 4 to 10.5 h. Peak serum concentration of Ki-4.dgA ranged from 0.23 to 1.7 µg/ml. In both trials ~60% of patients developed human anti-mouse and/or anti-dgA antibodies. Seventeen of 18 patients treated at the MTD of RFT5.dgA were evaluable for clinical response. Responses included two partial remissions (PR), one minor response (MR) and five stable diseases (SD). Responses included two partial remissions (PR), one minor response (MR) and five stable diseases (SD). Fifteen of 17 patients treated with Ki-4.dgA were evaluable for clinical response. Responses included one PR, one MR and two SD.

Conclusions: RFT5.dgA and Ki-4.dgA showed moderate efficacy in heavily pretreated refractory patients with HL. Ki-4.dgA was less well tolerated than RFT5.dgA. This might be due, at least in part, to the formation of Ki-4.dgA/sCD30 complexes.

Introduction

Hodgkin’s lymphoma (HL) has become a curable disease in many patients with polychemotherapy regimens such as MOPP or ABVD and improved radiation techniques [1–3]. More recently, patients with advanced disease have demonstrated improved responses and survival rates using the newly developed BEACOPP regimen [4]. Although most patients can be cured by standard approaches, fewer than 30% of those who relapse attain a durable disease-free remission after second-line treatment [5]. The outcome is even worse for those with primary refractory disease [6]. Data from patients with HL, as well as patients with other malignant diseases including colorectal cancer, myeloid leukemia and non-Hodgkin’s lymphoma (NHL), indicate that residual tumor cells remaining after first-line treatment can cause late relapses [7–11]. Thus, eliminating residual Hodgkin/Reed–Sternberg (HRS) cells after first-line treatment might improve the outcome in patients with HL. Approaches to eradicate residual tumor cells include monoclonal antibodies (mAbs) or mAb-based immunoconjugates [12, 13]. Since no effective ‘naked’ mAbs against HRS cells have been identified [14], immunotoxins (ITs) consisting of a specific cell-binding moiety and a potent toxin subunit were constructed. HL is a very suitable disease for IT treatment for several reasons. (i) HRS cells express surface antigens such as CD15 [15], CD25 [16] and CD30 [17]. These antigens are present only on a minority of normal human cells but not on stem cells. (ii) The number of HRS cells that must be killed is relatively small (<1% of lymphoid cells). (iii) The mechanism of cell destruction by ITs is different from that of conventional agents, thus circumventing drug resistance. (iv) ITs are capable of killing dormant non-dividing cells.

The lymphoid activation markers CD25 and CD30 are excellent targets for ITs in HL. Our group has tested mAbs against these antigens for their ability to make effective dgA-containing ITs [18]. The ITs were constructed by linking the mAb via a sterically hindered linker 4-succinimidyl-oxycarbonyl-α-methyl-α-(2-pyridyl)dithio) toluene (SMPT) to dgA. We conducted phase I/II trials using the two most active dgA-containing ITs, RFT5.dgA (CD25) and Ki-4.dgA (CD30) in patients with refractory HL [19–21].
Patients and methods

Patients

Patients with a histologically confirmed diagnosis of HL or anaplastic large cell lymphoma (ALCL) were entered into these trials. All patients were refractory to conventional therapy and had relapsed with evidence of progressive disease. To be eligible, patients had to be older than 18 years, and had to have measurable disease, a life expectancy of at least 3 months, a Karnofsky performance status of at least 50%, creatinine <2 mg/100 ml, sGPT <3 times the upper limit of normal, total bilirubin <1.5 mg/ml, albumin >75% of the lower limit of normal and a cardiac ejection fraction of ≥55%. In addition, >30% of the HRS cells had to stain positively for CD25 or CD30. Exclusion criteria were severe comorbidity, active second malignancy, chemotherapy or radiotherapy within 4 weeks, significant impairment of pulmonary function, granulocytes <1500/µl, platelets <20000/µl or any other major organ dysfunction unrelated to lymphoma, pregnancy, and presence of >1 µg/ml human anti-mouse antibodies (HAMA) or human anti-dgA antibodies (HARA). Concomitant administration of steroids was permitted with the same dose of steroids used for at least 4 weeks prior to enrollment.

Immunotoxins

The ITs RFT5.dgA and Ki-4.dgA were prepared as described [22] by coupling deglycosylated ricin A-chain (dgA; Inland laboratories, Austin, TX, USA) with the heterobifunctional crosslinker SMPT (Pierce, Rockford, IL, USA) to RFT5 or Ki-4, respectively. The ITs were formulated as a sterile solution containing 0.85% NaCl. Vials with 5 ml of IT at 0.5 mg/ml were frozen and maintained at -70°C. Before use, the IT was filtered through a 0.22 µm filter. The characteristics of both ITs are shown in Table 1.

Protocol design and regimen

RFT5.dgA and Ki-4.dgA were administered intravenously (i.v.) in 100 ml of isotonic saline over 4 h. The calculated total dose of the IT for one cycle of treatment was divided by four and administered once every 48h. We planned to treat cohorts of three patients with escalating doses of 5, 10, 15 and 20 mg/m²/course. This regimen was chosen based on prior experience in NHL patients [23]. We added a 7.5 mg/m² dose level in the trial with Ki-4.dgA due to high toxicity at the 10 mg/m² dose level. Patients were not entered at the next highest level until all three patients in the previous cohort had completed study day 14. If one patient experienced grade 3 or 4 toxicity, three additional patients were enrolled at that dose level. If three patients experienced grade 3 toxicity at one dose level, then the previous dose level was regarded as the maximum tolerated dose (MTD). If two patients at a dose level experienced a grade 3 toxicity and one patient at the next dose level a grade 4 toxicity, then the MTD was the dose at which the grade 3 toxicities occurred. If one patient experienced a grade 3 toxicity and another patient experienced a grade 4 toxicity at a dose level, then the previous dose level was defined as the MTD. The study design was in accordance with the Declaration of Helsinki. The Ethical Committees at the University of Cologne and the Institutional Review Board at the UT SWMC approved this trial. All patients gave written informed consent before treatment.

Assessment of toxicity

Adverse events were graded according to the WHO toxicity criteria as grade 1 (asymptomatic, easily tolerated), 2 (mild, tolerable), 3 (moderate, poorly tolerated) or 4 (severe, life threatening). Vascular leak syndrome (VLS) was specifically graded as described elsewhere [24]. Briefly, grade 1 was defined as minimal ankle pitting edema, grade 2 as ankle pitting edema and weight gain <7 kg, grade 3 as peripheral edema and weight gain 7–14 kg or pleural effusion without pulmonary dysfunction, and grade 4 as anasarca, pleural effusion or ascites with respiratory deficit or edema >14 kg.

Pharmacokinetics

To determine serum levels of intact RFT5.dgA or Ki-4.dgA in the peripheral blood of the patients, a RIA was used as previously described [25]. Half lives (t½) and areas under the curve (AUCs) were analyzed using the PKCALC program (developed by R. C. Shumaker; Merrel Dow Research Institute, Cincinnati, OH).

Flow cytometric analyses

Blood counts, and phenotyping (CD3, CD4, CD8, CD16, CD19, CD25, CD30, CD45RO) (FACScan; Becton Dickinson, Heidelberg, Germany) were performed before the first cycle of treatment and at least on days 4 and 9 of the first cycle, and before the second cycle.

Table 1. Characteristics and formulation of RFT5.dgA and Ki-4.dgA

<table>
<thead>
<tr>
<th>Immunotoxin</th>
<th>RFT5.dgA</th>
<th>Ki-4.dgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody subclass</td>
<td>IgG1</td>
<td>IgG1</td>
</tr>
<tr>
<td>Target antigen</td>
<td>IL-2 receptor (α-chain), CD25</td>
<td>CD30, cluster A</td>
</tr>
<tr>
<td>Endotoxin (Limulus amebocyte lysate assay) (endotoxin U/ml)</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>SDS–PAGE (% as M, 170 000 band)</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>A-chain activity relative to native dgA (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>IT binding activity relative to native antibody (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Crossreactivity</td>
<td>Activated lymphocytes</td>
<td>Modest staining with some activated cells in the pancreas, testis and epididymis, activated lymphocytes</td>
</tr>
<tr>
<td>IC50 on L540Cy cells (M)</td>
<td>7 × 10⁻¹²</td>
<td>4 × 10⁻¹¹</td>
</tr>
<tr>
<td>Effects in vivo</td>
<td>SCID mice (95% CCR)</td>
<td>SCID mice (50% CCR)</td>
</tr>
</tbody>
</table>

IT, immunotoxin; IC50, inhibition concentration of 50%; CCR, continuous complete remission; SCID, severe combined immunodeficiency.
Soluble antigens

Soluble circulating antigens were analyzed in patients in both trials by standard ELISA methods: sCD30 (DAKO, Hamburg, Germany) and sCD25 (R & D Systems, Minneapolis, MN, USA). Analyses were performed on study days 0, 1, 3, 6 and 10. To quantify levels of unbound sCD30 after treatment with Ki-4.dgA, we used a modified DAKO ELISA Kit. This test system is based on plates coated with mAb Ki-1, which is directed against cluster B of the CD30 antigen. The peroxidase-conjugated mAb, Ber-H2, is directed against cluster A of the CD30 antigen. This cluster is also recognized by Ki-4. Thus, we performed modified ELISA using the coating mAb Ki-1 and the peroxidase-conjugated mAb Ki-3, which binds to cluster C and does not interfere with Ki-4. The concentration of sCD30/Ki-4.dgA complexes was evaluated indirectly by subtracting the value of the standard DAKO ELISA from the value of the modified ELISA.

Natural killer cell activity

Since the CD25 antigen is expressed on a small subset of natural killer (NK) cells we analyzed the NK cell activity of all patients treated with RFT5.dgA using an europium-DTPA (Eu-DTPA) assay as described previously [26, 27]. NK cell activity was defined as percentage of the maximum europium release of the NK cell sensitive cell line K562 after incubation with 0.4 × 10^6 peripheral blood mononuclear cells (PBMCs). NK cell activity was determined on study days 0, 2 and 9, and before the second cycle, and compared with the NK cell activity of healthy matched probands.

Detection of HAMA and HARA

HAMA and HARA were measured before treatment and on day 10 of each cycle as described previously [25].

Evaluation of response

Restaging, including computed tomography scans of involved areas, was performed 28–35 days after completion of treatment. WHO criteria for responses were used. A minimum of two cycles was required to evaluate treatment efficacy, unless there was rapid progression. Responding patients were eligible for retreatment if they had <1 µg/ml HAMA and HARA prior to the next cycle of treatment.

Statistical analysis

Demographics and disease characteristics were summarized for all patients using descriptive statistics. Statistical significance was compared using the t-test or Wilcoxon rank sum test.

Results

Patients

Table 2 summarizes the demographics of all patients treated. In general, characteristics of the 27 patients treated with RFT5.dgA and the 17 patients receiving Ki-4.dgA were relatively similar. The median age was 30.5 years (range 19–47 years) in the RFT5.dgA trial and 35 years (range 24–52 years) in the Ki-4.dgA trial. In both studies >80% of the patients had advanced disease. Primary progressive disease was observed in 10/27 versus nine of 17 patients. Most patients were heavily pretreated with an average of four versus six different chemotherapies (range two to eight versus two to nine) including high-dose chemotherapy and autologous bone-marrow transplants in 13 patients in each trial.

Table 2. Demographics of patients treated with RFT5.dgA and Ki-4.dgA

<table>
<thead>
<tr>
<th></th>
<th>RFT5.dgA</th>
<th>Ki-4.dgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Age, years (range)</td>
<td>30.5 (19–47)</td>
<td>35.0 (24–52)</td>
</tr>
<tr>
<td>Stage at study entry</td>
<td>II A</td>
<td>II A</td>
</tr>
<tr>
<td>IIA</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>IIIA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IIIB</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>IVA</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>IVB</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>MC</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>LP</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ALCL</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Primary refractory</td>
<td>10/27</td>
<td>9/17</td>
</tr>
<tr>
<td>Prior chemotherapies (range)</td>
<td>4 (2–8)</td>
<td>6 (2–9)</td>
</tr>
<tr>
<td>ABMT/PSCT</td>
<td>13/27</td>
<td>13/17</td>
</tr>
</tbody>
</table>

According to the Ann Arbor classification system. NS, nodular sclerosis; MC, mixedcellularity; LP, lymphocyte predominant; ALCL, anaplastic large cell lymphoma; ABMT, autologous bone marrow transplantation; PSCT, peripheral stem cell transplantation.

Toxicity

Table 3 lists all adverse events of patients in both trials according to standard WHO criteria. Dose-limiting toxicities (DLTs) were VLS, fatigue and myalgia. The most frequent side effects observed in both trials were VLS (27/27 and 16/17), fatigue (26/27 and 15/17), myalgia (20/27 and 10/17), nausea/vomiting (14/27 and five of 17), tachycardia (11/27 and 17/17), hypertension (13/27 and 15/17), joint pain or diffuse pain (seven of 27 and 10/17), and skin reaction including mild erythema and desquamation (four of 27 and 10/17). Hematological parameters (leukocytes, hemoglobin, thrombocytes) were stable with the exception of one grade 2 thrombopenia in the RFT5.dgA trial. The MTDs for RFT5.dgA and Ki-4.dgA were 15 and 5 mg/m², respectively.

Pharmacokinetics

In all patients, the Cmax was reached at the end of the IT infusion, returning to or close to baseline after 12–24 h. The Cmax correlated only roughly to the administered dose (correlation coefficient 0.68). The Cmax achieved in the RFT5.dgA trial ranged from 0.2 to 9.7 µg/ml and in the Ki-4.dgA trial from 0.23 to 1.1 µg/ml. The maximum t½s for RFT5.dgA ranged from...
Flow cytometric analysis of PBMCs

Of the antigens analyzed on PBMCs, only the CD25+ cells demonstrated significant changes during treatment with RFT5.dgA. CD25/CD3+ and CD25/CD4+ PBMCs showed a significant decrease (P < 0.001) of mean fluorescence intensity immediately after the initiation of IT therapy, which persisted during therapy and recovered thereafter. The reduction of CD25+ cells probably reflects a destruction of CD25+ cells by the IT or, alternatively, a modulation of CD25, since the mAb used for the FACS analysis of CD25 (anti-TAC) is cross-blocked by the mAb used for construction of the IT. CD25/CD8+ and CD25/CD19+ PBMCs were much less affected. This might be in part due to the lower mean fluorescence intensity of these cells as compared with that of the CD25/CD3+ and CD25/CD4+. In the Ki-4.dgA trial, CD30+ PBMCs were not measured.

Cytokines and soluble antigens

sCD30 was detected in 15/15 patients of the RFT5.dgA phase I trial (median 133 U/ml; range 3–952 U/ml) and in 16/17 of the patients in the Ki-4.dgA trial (median 101 U/ml; range 0–623 U/ml). Patients who had a less rapidly growing tumor before enrollment and relatively small tumor burden had concentrations of sCD30 of <100 U/ml.

Twenty-four hours after the first infusion of Ki-4.dgA, sCD30 was no longer detectable using the conventional DAKO ELISA kit with the peroxidase-conjugated mAb Ber-H2, which is directed against the same CD30 cluster as Ki-4. In contrast, this effect was not observed when using a modified ELISA with the peroxidase-conjugated mAb Ki-3, which is directed against a distinct CD30 cluster. Thus, sCD30 was non-competitively targeted by Ki-4.dgA and persisted in the peripheral blood. Binding of RFT5.dgA to sCD25 was not observed.

Table 3. Side effects of RFT5.dgA and Ki-4.dgA

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of pts</th>
<th>WHO grade 3 and 4 toxicity* [character (no. of pts)]</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFT5.dgA trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>None</td>
<td>1 MR, 1 SD, 1 PD</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>None</td>
<td>1 SD, 2 PD</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>5 pts: grade 3 [fatigue (3), VLS (2), dyspnea (2), myalgia (2)]</td>
<td>2 PR, 1 MR, 5 SD, 9 PD, 1 NE</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>3 pts: grade 3 [nausea (2), fatigue (2), VLS (1), tachycardia (2), dyspnea (2)]; grade 4 [myalgia (1)]</td>
<td>3 PD</td>
</tr>
<tr>
<td>Ki-4.dgA trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>1 pt: grade 3 [myalgia (1)]</td>
<td>1 PR, 1 MR, 1 SD, 6 PD</td>
</tr>
<tr>
<td>7.5</td>
<td>6</td>
<td>2 pts: grade 3 [VLS (1)]; grade 4 [myalgia (1)]</td>
<td>4 PD, 2 NE</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>2 pts: grade 3 [fatigue (1), VLS (2), dyspnea (1), myalgia (1)]</td>
<td>1 SD, 1 PD</td>
</tr>
</tbody>
</table>

*More than one toxicity occurred in some patients.

Concentrations of sCD25 (median 2265 U/ml, range 512–14940 U/ml; versus median 6340 U/ml, range 3034–15 000 U/ml, normal 244–560 U/ml) were high in all patients except in two. Patients in the Ki-4.dgA trial whose sCD25 levels were stable or decreased had a favorable clinical course with stable disease (SD) or only mild tumor progression, whereas the patients with levels of sCD25 >10 000 U/ml progressed rapidly.

NK cell activity

The NK cell activity of the patients treated with RFT5.dgA ranged from 5.5% to 100% tumor cells lysis with a median of 31%, whereas the NK cell activity of 10 probands was significantly higher (P <0.001), ranging from 22% to 95% (median 52%). Only on study day 9 was the NK cell activity of the patients significantly lower (median 21%; P = 0.018) compared with the NK cell activity before treatment. Complete recovery of NK cell activity was reached before start of the second treatment cycle.

Development of HAMA and HARA

No patient made HAMA and/or HARA after the end of the first cycle. In the RFT5.dgA trial HAMA and HARA responses >1 µg/ml developed in 14/22 (64%) patients receiving two or more cycles of IT. The development of HAMA and/or HARA had a significant influence on the pharmacokinetics of the IT in the second cycle of treatment. Thus, in patients with HAMA/ HARA no unbound IT was detectable (Figure 1).

Of 10 patients receiving two or more cycles of Ki-4.dgA, 60% had a significant HARA response, whereas only 10% made significant amounts of HAMA.

Clinical response

Twenty-six of 27 patients receiving RFT5.dgA were evaluable for clinical response. Two partial remissions (PRs), two minor responses (MRs) and seven SDs were documented. The PRs occurred at MTD. The PRs lasted 2 and 25 months, respectively. Of 15 evaluable patients in the Ki-4.dgA trial, one PR, one MR
and two SDs were documented. The PR lasted 5 months. One patient had a regression of neurological symptoms caused by intraspinal HL for 3 weeks, but without measurable tumor regression.

Discussion

Both ITs, RFT5.dgA and Ki-4.dgA demonstrated impressive pre-clinical activity against human HL in vitro as well as in mouse models [28, 29]. Based on these results we performed clinical trials with these ITs. In these studies, we evaluated the MTD, the DLT and response rate in patients with refractory HL. The major findings are as follows. (i) The MTDs of RFT5.dgA and Ki-4.dg were 15 and 5 mg/m², respectively. (ii) The DLTs were related to VLS including hypoalbuminemia, weight gain, tachycardia, hypotension, dyspnea, weakness and fatigue. Other side effects were myalgia and nausea/vomiting. (iii) Responses in this group of heavily pretreated patients included two PRs and two MRs for the RFT5.dgA trial, and one PR and one MR for the Ki-4.dgA trial. (iv) About 60% of patients made HARA and/or HAMA >1.0 µg/ml.

The toxicity profile, response rates and C_{max} of the ITs used in our trials are similar to those observed with other IgG.dgA-containing ITs in patients NHL. In patients with relapsed NHL receiving the anti-CD22 A-chain IT, Fab'-RFB4.dgA, in a schedule identical to our trials, five of 14 (36%) PRs were observed [25]. Administration of the IgG IT (IgG-RFB4.dgA) in a similar NHL patient group resulted in one complete remission (CR) and five PRs (overall 25%) in 24 evaluable patients [23]. In a subsequent phase I study, RFB4.dgA given as continuous infusion over 8 days induced comparable clinical results (four of 18 PR, 22%) and toxicity [24]. In another trial with the anti-CD19 IT, HL37.dgA, one CR and one PR occurred in 23 evaluable patients (overall 9%) on the bolus regimen (MTD 16 mg/m²) compared with one PR (11%) in nine patients treated with the continuous infusions (MTD 19 mg/m²) [30]. Peak serum concentrations of the IT at MTD were also similar. An analysis of all NHL patients treated in clinical phase I/II protocols with dgA-containing ITs suggested that toxicity is more frequent and more severe in patients with prior irradiation [31]. In these studies, as well as in our trial, DLT was related to VLS. In contrast to the trials in NHL patients, we observed neither pulmonary edema nor aphasia related to VLS. Since >50% of the patients in our trials had pulmonary HL, other reasons must be considered. The most obvious explanation, apart from the different histology, is the younger age of patients enrolled in our trials (30–35 years, compared with 49–60 years in the NHL trials). Thus, this suggests that different symptoms of VLS might be prevalent in patients of different ages, that HL and NHL have different predisposing factors which have not yet been identified.

VLS occurred at lower doses in the Ki-4.dgA trial than in the RFT5.dgA trial and similar trials with other ITs in patients with NHL. This might be related to the small numbers of CD30+ PBMCs, the binding of Ki-4.dgA to sCD30 and the lack of shed antigens such as CD19 and CD22 in NHL patients. A strong inverse correlation between circulating tumor cells and toxicity has been reported in other trials [32]. In the RFT5.dgA trial, binding of RFT5.dgA to sCD25 was not detected, even though it bound to CD25+ PBMCs. To reduce binding of Ki-4.dgA to sCD30 it might be prudent to infuse the native mAb before treatment with the IT. Since metalloproteinases induce the shedding
of CD30, the blockade by hydroxamic acid-based metalloproteinase inhibitors might also reduce toxicity (H. P. Hansen, B. Matthey, P. Borchmann, R. Schnell, S. Tawadros, H. Lange, M. Huhn, A. Klimka, S. Barth and A. Engert, submitted). VLS results from dgA-mediated damage of endothelial cells [33]. The demonstration of a three amino acid sequence motif in ricin A-chain, IL-2, Pseudomonas exotoxin and diphtheria toxin as the binding site might allow genetic engineering of constructs to avoid VLS [34]. Such an A-chain mutant has been constructed showing encouraging results (E.S.Vitetta, unpublished).

In a clinical trial using a different anti-CD30 IT consisting of the mAb Ber-H2 coupled to Saporin-S6, a single-chain ribosome-inactivating protein (type 1 RIP), four patients with refractory HL were treated with 0.2–0.8 mg/kg Ber-H2-Sap6 in one or two infusions over 4 h [35]. Within 5–7 days, substantial decreases in tumor mass were observed in three of four patients, lasting 6–10 weeks. Toxicity included fever, malaise, anorexia, fatigue, mild myalgias, weight gain and a reversible 4- to 5-fold increase in liver enzymes. Eight patients were subsequently enrolled [36]; of 12 patients treated overall, four achieved PRs and three MRs with a median duration of 2 months. The MTD of 0.8 mg/kg was established by grade 3 reversible VLS and liver toxicity.

About 60% of patients in our IT trials developed HAMA and HARA, which is limiting of the number of courses of the IT [37] and influences the pharmacokinetics. Development of HAMA/ HARA might be reduced by the use of humanized [38] or recombinant constructs with human ligands [39] or toxins [40].

Future strategies in the use of ITs could include IT ‘cocktails’, i.e. a combination of mAb-based constructs directed against different antigens on a tumor cell. A ‘cocktail’ of two or more ITs against different antigens (CD25, CD30, IRac) on HRS cells had superior effects in vitro and in nude mice compared with single ITs [35]. Similar results were reported against Daudi lymphoma cells in vitro and in severe combined immunodeficiency (SCID) mice [41]. This led to a study in which 22 patients with refractory NHL were treated with a continuous infusion of a combination of IgG-307.dgA and IgG-RFB4.dgA at doses of 10–30 mg/m² (MTD 10 mg/m²). Two PRs (9%) and five MRs were reported [32]. Another approach might be to use recombinant constructs. Several recombinant ITs (rIT) against CD25 have been evaluated in clinical trials. LeMaistre et al. [42, 43] conducted two studies with the diphtheria toxin-based DAB₄₈₉IL-2 in hematological malignancies expressing the IL-2 receptor. No response occurred in four patients with HL. In another trial, one CR lasting >2 years in one of four HL patients was achieved with DAB₄₈₉IL-2 [44]. The MTD of 0.2 µg/kg daily was determined by hypersensitivity-like symptoms and reversible elevation of transaminases on higher dose levels. In contrast to their earlier phase I data, none of 17 HL patients responded to DAB₄₈₉IL-2 in a phase II trial including 73 patients with different lymphomas [45]. The MTD was 27 µg/kg/day. Fatigue was the DLT. Other toxicities included fever, chills and nausea. In a clinical phase I trial using the anti-CD25 rIT anti-Tac(Fv)-PE38 (LMB-2), derived from Pseudomonas exotoxin, 11 patients with refractory HL were treated with three doses of 2–63 µg/kg i.v. on alternate days [46]. The MTD was 40 µg/kg with transient transaminase elevations and fever. Five of 11 patients made HAMA and seven of 11 patients made antibodies against PE38. Responses include one PR, three MRs and six SDs. Our group generated anti-CD25 and anti-CD30 rITs, which have shown high efficacy in vitro and in SCID mice with human Hodgkin’s tumors [47–49].

In conclusion, RFT₅.dgA and Ki-4.dgA given to patients with resistant HL demonstrated moderate tolerability and efficacy. This might be related to the unfavorable patient selection in our studies since patients were pre-treated with multiple prior chemotherapies. They had highly active disease and large tumor burden. Reduction of toxicity and administration of higher doses might be feasible by the use of new constructs that do not cause VLS. The high percentage of antibody response should be reduced by the use of human or humanized rITs allowing repetitive cycles of treatment. Thus, future clinical trials should evaluate whether modified ITs can be validated in HL patients.

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References


