CD30 is an excellent target for immunotherapy of Hodgkin lymphoma (HL) because it is overexpressed on Hodgkin and Reed-Sternberg cells. We developed a novel bispecific molecule (BSM) consisting of F(ab')2 fragments derived from the murine anti-CD30 monoclonal antibody (MoAb) KI-4 and the humanized CD64-specific MoAb H22. In vitro experiments of H22xKi-4 demonstrated specific phagocytosis of HL-derived cell lines. Patients (pts) with refractory CD30+ HL were treated with escalating doses of H22xKi-4 at doses of 1, 2.5, 5, 10, and 20 mg/m²/d, respectively (administered intravenously on days 1, 3, 5, and 7). The main study objectives were to determine the maximum tolerated dose and the dose-limiting toxicities of H22xKi-4, to define its pharmacokinetic profile, and to document clinical response. Ten pts were enrolled and are evaluable for toxicity and response. Side effects were transient and mild with hypotension (4 of 10), tachycardia (6 of 10), fatigue (10 of 10), and fever (2 of 10 grade I, 3 of 10 grade II). Pharmacokinetic (PK) data revealed an elimination half-life of 11.1 hours, resulting in a significant accumulation of H22xKi-4. The BSM was shown to bind to both monocytes and malignant cells. Response to H22xKi-4 included 1 complete remission (CR), 3 partial remissions (PR), and 4 pts with stable disease. The new BSM H22xKi-4 can be given safely to pts with refractory CD30+ HL in doses up to 80 mg/m² per cycle. Although this dose is not the maximum tolerated dose (MTD) as defined by toxicity criteria, surrogate parameters suggest a biologic effective regimen. H22xKi-4 shows activity in heavily pretreated HL patients warranting further clinical evaluation. (Blood. 2002;100:3101-3107)

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Introduction

Hodgkin lymphoma (HL) has become a curable disease after the introduction of polychemotherapy regimens such as MOPP (mechlorethamine, vincristine, procarbazine, prednisone) or ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) and improved radiation techniques. More recently, patients (pts) with advanced-stage disease show improved response and survival rates using the BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) regimen established by the German Hodgkin’s Lymphoma Study Group. Although most pts can be cured by standard approaches, fewer than 30% of those who relapse attain a durable disease-free remission after second-line treatment. The outcome is even worse for those with primary refractory disease. Data from HL as well as from other malignant diseases, including colorectal cancer, myeloid leukemia, or non-Hodgkin lymphoma (NHL), suggest that small numbers of residual tumor cells remaining after first-line treatment can give rise to late relapses. Thus, eliminating residual Hodgkin–Reed-Sternberg (H-RS) cells after first-line treatment might further improve outcome in HL. Monoclonal antibody (MoAb)–mediated cell lysis may be ideally suited to eliminate residual tumor cells in HL for several reasons: (1) H-RS cells consistently express high amounts of potential target antigens such as CD25 and CD30, (2) human Hodgkin lymphoma contains only a minority of malignant H-RS cells and are well vascularized, and (3) the mechanism of cell kill and side effects of antibody-mediated cell lysis are completely different from conventional therapy. Among the different target antigens on H-RS cells, CD30 seems to be the most promising, because it is strongly overexpressed in Hodgkin lymphoma. A number of different MoAbs have been evaluated for treatment of HL pts, including antibody-toxin constructs (immunotoxins), radioimmunoconjugates, and unmodified MoAbs. As a possible alternative, bispecific antibodies (BsAbs) have attracted interest as immunoreagents in HL. In general, BsAbs have been shown to be well tolerated. However, side effects and cytotoxic potential of these constructs crucially depend on the effector cells targeted. So far, most BsAbs involved different subsets of lymphocytes or natural killer (NK) cells, which might be less effective in pts with malignant disease and, in particular, HL. Thus, we constructed a new bspecific molecule (BSM) based on the high-affinity FcγRI receptor. CD64 is part of this receptor and is expressed on activated neutrophils, monocytes, and macrophages. CD64 serves as a trigger molecule for cytotoxic effector cells expressing FcγRI. Both monomeric immunoglobulin G (IgG) as well as IgG-antigen complexes bind to FcγRI. Binding of only IgG-antigen complexes...
to FcγRI results in increased cytotoxic activity, including cytolysis, respiratory burst, and production of oxidative enzymes. The murine MoAb M22 binds to the FcγRI at an epitope outside the normal Fc binding domain, thereby circumventing the competition for murine MoAb M22 binds to the Fc/H9253 derived macrophages (MDM). Thus, CD64 is a promising target for monocytes as well as phagocytosis in conjunction with monocyte-derived antibody-dependent cellular cytotoxicity (ADCC) in conjunction with monocytocytic cells as well as phagocytosis in conjunction with monocyte-derived macrophages (MDM). Thus, CD64 is a promising target for the recruitment of immunocompetent effector cells in HL. Here we report the results of a clinical phase 1 study designed as “proof of principle” of this novel therapeutic BSM in pts with HL.

Patients and methods

Patients

Eligible pts had measurable and active advanced refractory HL not amendable for conventional chemotherapy. Presence of the CD30 antigen had to be documented by reactivity with anti-CD30 antibodies on at least 30% of H-RS cells obtained from tumor biopsy performed within 1 year before treatment with H22xKi-4. Prior chemotherapy or radiotherapy had to be completed 4 weeks before study drug administration. In addition, the following conditions had to be fulfilled: presence of objectively measurable sites of disease, World Health Organization (WHO) performance status of 2 or less, age between 18 and 70 years, life expectancy of at least 3 months, serum creatinine of less than 177 μM (2 mg/100 mL), serum albumin of more than 75% of the lower limit, cardiac function as measured by echocardiography with a baseline left ventricular ejection fraction (LVEF) more than 35%, and no other major medical problems. Concomitant corticosteroid treatment was not an exclusion criterion because pts with progressive HL often require corticosteroid therapy. The protocol was approved by the institutional ethics committee, and pts had to give written informed consent as to the investigational nature of the treatment prior to entry into the study.

Study design

This clinical trial was an open-label, nonrandomized, phase 1, multiple-dose escalation study. The primary objective was to determine the maximum tolerated dose (MTD) and the dose-limiting toxicity (DLT) of H22xKi-4 in humans when administered by intravenous infusion. Secondary objectives included the pharmacokinetics, the biologic optimum dose, and possible antitumor activity. Pts received at least 2 courses of treatment each consisting of 4 intravenous infusions of H22xKi-4 administrated on days 1, 3, 5, and 7. The second course was started on day 21. Additional courses were used to determine the pharmacokinetic parameters and possible antitumor activity. Pts received an initial test dose of either 10% of the total dose or 0.2 mg, whichever was smaller, of H22xKi-4, dissolved in 50 mL normal saline and administered intravenously over 10 minutes. Pts were then premedicated with 1000 mg acetaminophen orally and 1 mg clemastine orally 30 minutes prior to receiving the final dose of H22xKi-4. If this test dose was tolerated without any significant toxicity after 30 minutes, H22xKi-4 was diluted in 500 mL normal saline and administered intravenously starting with 3 mg/h. If no adverse reactions were noted after 60 minutes, the infusion rate was increased to 6 mg/h and then to 9 mg/h, respectively.

Pharmacokinetics

On the first day of H22xKi-4 administration, blood samples were drawn in heparinized tubes at the following time points: preinfusion, immediately at the end of the infusion, and at 2, 4, 8, 12, 24, and 48 hours following the end of the infusion. Plasma was separated from blood cells by spinning at 1,200g for 10 minutes and subsequently stored at −20°C until analysis of H22xKi-4 concentrations. Microtiter plates coated with goat anti–murine IgG were incubated with dilutions of patient plasma or H22xKi-4 prepared in normal human plasma (Nabi, Boca Raton, FL). The captured BSM was detected by an alkaline phosphatase-conjugated goat anti–murine IgG probe. The means of duplicate determinations of patient plasma dilutions were used to determine H22xKi-4 concentration from the linear portion of the standard curve. The limit of sensitivity for accurate measurement was 0.125 mg/L for the first 3 pts and 0.04 mg/L for all subsequent pts.

Pharmacokinetic analysis

The data for the plasma H22xKi-4 concentration over time were inspected on a semilogarithmic plot of H22xKi-4 concentration versus time for each subject. The Cmax and Tmax values were the values observed from the raw pharmacokinetic data. The other standard pharmacokinetic parameters were estimated using the WinNonlin Pro pharmacokinetic program (Pharsight, Mountain View, CA). The concentration–time data were analyzed using an open noncompartmental method (WinNonlin model 202). The terminal elimination rate constant (k) was determined by noncompartmental analysis using a linear regression of the terminal 3 to 6 points of the log plasma H22xKi-4 concentration versus time plot using a nonweighted paradigm. The terminal elimination half-life (τ1/2ₚ) was estimated from 0.693/k. The area under the curve (AUC) to the last datum point was estimated using the linear-trapezoidal rule and extrapolated to infinity by adding the Wagner-Nelson correction (Cmax/k). Total body clearance (CL)
was calculated by dividing the dose by $AUC_{(0-\infty)}$. The apparent volume of distribution ($V_{dz}$) was estimated from $CL_{pK}$. The mean residence time (MRT) was estimated from AUMC/AUC. The accumulation factor $R$ was estimated from the equation $Treat_{4}\cdot AUC_{(0-\infty)}/Treat_{1}\cdot AUC_{(0-\infty)}$. In this Treat 4, $AUC_{(0-\infty)}$ was the AUC from zero to the dosing interval on treatment occasion 4 (day 7), and $AUC_{(0-\infty)}$ was the AUC from zero to infinity on day 1.

**Evaluation of biologic activity**

Because DLTs with this BSM were not likely to occur, surrogate parameters for the biologic activity were investigated. Monocyte counts in the peripheral blood were measured immediately before and after infusion of $H_{22}x{K}_{i-4}$ and at 2, 4, 8, and 24 hours after infusion. CD64 expression on peripheral blood monocytes as determined by CD14 positivity was determined by fluorescence-activated cell sorter (FACS) analysis at indicated time points and correlated to an isotype control using an irrelevant murine antibody (FACScalibur, Becton Dickinson, NJ). CD64 index was calculated as follows: mean fluorescence intensity (MFI) $CD64$ (monocyte gating)/MFI isotype control (monocyte gating).

With regard to the tumor, $sCD30$ concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark) before and after each day of BSM administration. Two pts gave informed consent for a diagnostic biopsy of enlarged peripheral lymph nodes 24 hours after the last infusion of $H_{22}x{K}_{i-4}$. This material was divided into 2 parts, one of which was immediately fresh frozen and stored at $-80^\circ C$ and the other embedded in paraffin. For immunohistochemical investigation, the tissue was deparaffinized, cut into sections of 5 μm, and blocked with pig serum for 10 minutes to reduce unspecific staining. Then the primary MoAb, a polyclonal rabbit antimouse Ab (DAKO), diluted 1:50 in phosphate-buffered saline (PBS) was applied and incubated at 4°C overnight, followed by a biotinylated pig antirabbit antibody (1:200 for 45 minutes at room temperature; E 431 DAKO) and a standard biotin-streptavidin kit (DAKO). Finally, the slides were stained with fast-red (DAKO). As a first negative control (BSM-free), a specimen of HL from a patient who had not been treated in this study was stained during the same procedure. A second negative control was stained without the primary antibody to exclude unspecific cross-reactivity from antibodies used for the staining procedure. Due to the nature of these investigations, there was no positive control available.

**Human antibispecific antibody (HABA) and human antimouse antibody (HAMA) response**

Human antibispecific antibody response was determined using a method described previously. Briefly, microtiter plates coated with the BSM were incubated with dilutions of plasma samples and anti-BSM antibodies detected with an alkaline phosphatase–conjugated goat-anti–human IgG Fc-specific probe. HABA levels were expressed as x-fold increase over the baseline preinfusion value.

**Statistical methods**

Changes in pharmacokinetic parameters over time (duration of the study treatment) were investigated using a one-way univariate repeated measures analysis of variance (MANOVA). The CD64 index was compared immediately before and after each day of BSM administration. Two pts gave informed consent for a diagnostic biopsy of enlarged peripheral lymph nodes 24 hours after the last infusion of $H_{22}x{K}_{i-4}$. This material was divided into 2 parts, one of which was immediately fresh frozen and stored at $-80^\circ C$ and the other embedded in paraffin. For immunohistochemical investigation, the tissue was deparaffinized, cut into sections of 5 μm, and blocked with pig serum for 10 minutes to reduce unspecific staining. Then the primary MoAb, a polyclonal rabbit antimouse Ab (DAKO), diluted 1:50 in phosphate-buffered saline (PBS) was applied and incubated at 4°C overnight, followed by a biotinylated pig antirabbit antibody (1:200 for 45 minutes at room temperature; E 431 DAKO) and a standard biotin-streptavidin kit (DAKO). Finally, the slides were stained with fast-red (DAKO). As a first negative control (BSM-free), a specimen of HL from a patient who had not been treated in this study was stained during the same procedure. A second negative control was stained without the primary antibody to exclude unspecific cross-reactivity from antibodies used for the staining procedure. Due to the nature of these investigations, there was no positive control available.

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**Results**

**Patients’ characteristics**

A total of 10 multiple pretreated relapsed HL pts treated on 5 different dose levels were included and are evaluable, of whom 2 were female (Table 1). The median age was 34.6 years (range, 21 to 53). Histology included 3 pts with mixed cellularity of Hodgkin lymphoma and 7 pts with the nodular sclerosis subtype. The median number of relapses was 3 (range 1 to 7). A median of 4 prior chemotherapies had been administered (range, 2 to 6), including high-dose chemotherapy with autologous stem cell support in 9 of 10 pts. In addition, all pts had been pretreated with radiotherapy. Time interval to the previous chemotherapy or radiotherapy was very short, with 2 to 7 months for all but one patient (no. 5, 18 months). Only 2 patients were on prednisone at study entry (nos. 1 and 10). Seven pts had stage IV and 3 pts stage III disease. Six had B symptoms on study entry. Eight pts were treated with 2 courses of $H_{22}x{K}_{i-4}$, 1 pt received 3, and 1 pt 4 courses (consisting of 4 infusions each course) of treatment, respectively.

**Toxicity**

All side effects were transient, occurring during and up to 6 hours after the end of the infusion (Table 2). In all 10 pts, mild fatigue was observed. Other toxicities included mild hypotension (4 grade I), tachycardia (6 grade I), fever (2 grade I, 3 grade II), chills (4 grade I), and myalgia (3 grade I). All of these side effects resolved within 24 hours of the BSM infusion. Neither hematologic nor organ toxicities were observed.

**Pharmacokinetics**

$H_{22}x{K}_{i-4}$ concentrations were detectable only in those pts receiving more than 5 mg/m²/d. Tₘax occurred at or after the end of the infusion in all subjects on all treatment days. The plasma $H_{22}x{K}_{i-4}$ concentration decay over time was monoexponential for all pts. There was a trend for Cₘax and AUC to increase over time. Therefore, a univariate, single-factor, repeated measures analysis of variance for Cₘax, T₁/₂z, AUC, Vdz, and Cl over the 4-treatment 7-day period was performed on the 6 pts in the 80 mg/m² dose cohort. This analysis revealed no significant effect of time on the elimination half-life (F = 0.153; P = .926), apparent volume of distribution (F = 0.179; P = .193), or clearance (F = 1.54; P = .254). There was a significant time effect on Cₘax (F = 6.38; P = .005) and AUC (F = 5.78; P = .008), which was primarily explained by a linear effect over time. This was suggestive of a slight accumulation of $H_{22}x{K}_{i-4}$ with repeated dosing (Figures 1 and 2). For all pts with available data, the median value of the accumulation factor R was 1.36 (range, 0.98-3.90) by the fourth treatment on day 7. The $H_{22}x{K}_{i-4}$ terminal half-life was 7.9 hours at the 10 mg/m²/d dose (n = 1) and had a mean value of 11.1 hours at the 20 mg/m²/d dose level (median 11.1 hours; range, 5.3 to 18.2 hours) (Table 3). The volume of distribution ranged from 20.26 to 183.20 L/m². The mean value of the apparent volume of distribution (Vdz) in the 20 mg/m² group was 53.17 L/m². The total body clearance of $H_{22}x{K}_{i-4}$ on day 1 varied from 1.02 to 14.06 L/h/m², with a mean value for the group of pts who received 20 mg/m² of 3.91 L/h/m² (SD 5.04 L/h/m²). Low titers of HABA were detectable after the end of the second course in all pts with measurable BSM levels, neither resulting in decreased serum concentrations of the BSM nor in allergic reactions (Table 2). The patient treated with 4 cycles of the BSM developed high HABA levels.
There was a decrease of the CD64 expression on peripheral blood monocytes as well as a decline of their blood counts (Figure 3). Serum sCD30 levels were markedly elevated in pts with a high tumor burden but were no longer detectable after the first infusion of the BSM and remained at very low levels until the end of treatment in all pts (Table 2).

Immunohistochemistry

The murine fragment of the BSM could be detected in the lymph node specimen taken from pt nos. 5 and 8 using the above-described method (Figure 4). There was bright staining of the H-RS cells that was located throughout the cytoplasm. In addition, macrophages in this tissue showed an identical staining pattern. Thus, there was clear evidence for penetration of the BSM into lymph nodes from pts with active HL.

Tumor response

Overall, there were 4 pts with objective responses to the H22xKi-4 BSM. One CR was seen in a patient with diffuse pulmonary nodules up to a maximum of 10 mm. This response lasted for 3 months, and then the pulmonary nodules became measurable again.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Histology</th>
<th>Stage</th>
<th>No. of prior chemotherapies</th>
<th>Time to last therapy preceding BSM, mo</th>
<th>No. of relapses</th>
<th>Dose, mg/m²/d</th>
<th>No. of courses</th>
<th>Involvement</th>
<th>Response</th>
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<tr>
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<td>26</td>
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<td>4</td>
<td>Thoracic vertebrae 5-8</td>
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<tr>
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<td>Mediastinal, axillary, abdominal LN, vertebral</td>
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</table>

M indicates male; F, female; MC, mixed cellularity; NS, nodular sclerosis; LN, lymph node; PD, progressive disease; SD, stable disease; PR, partial remission; and CR, complete remission.

Biologic activity

There was a decrease of the CD64 expression on peripheral blood monocytes as well as a decline of their blood counts (Figure 3). Serum sCD30 levels were markedly elevated in pts with a high tumor burden but were no longer detectable after the first infusion of the BSM and remained at very low levels until the end of treatment in all pts (Table 2).

Table 2. Soluble CD30 and toxicities of H22xKi-4

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose level, mg/m²/d</th>
<th>sCD30 pretherapy, U/mL</th>
<th>sCD30 after second course, U/mL</th>
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<th>Hypotension, grade</th>
<th>Fever, grade</th>
<th>Chills, grade</th>
<th>Myalgia, grade</th>
<th>Fatigue, grade</th>
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* X-fold increase at the end of therapy compared with baseline.
by computed tomography (CT) scan and a rescue chemotherapy was initiated. PR was documented in 3 pts lasting from 4 weeks to 5 months. One pt (no. 4) had additional chemotherapy after 4 weeks. In this patient, the only site of the disease was a thoracic vertebrae 5-8 in disease, and 4 pts showed stable disease. Of these, 1 pt (no. 6) with advanced and refractory HL. H22xKi-4 is a new bispecific molecule consisting of 2 chemically linked Fab’ fragments derived from the murine anti-CD30 MoAb Ki-4 and the humanized anti-CD64 MoAb H2. This construct had shown activity against H-RS cells, suggesting an effective dose and schedule.

Two pts treated at the lowest 2-dose cohorts had progressive disease, and 4 pts showed stable disease. Of these, 1 pt (no. 6) with massive tumor burden (infiltration of the right upper lung with pleural and thoracic wall infiltration), who had experienced life-threatening toxicities (sepsis, acute renal failure, mechanical ventilation for 2 months) upon preceding chemotherapy, achieved a marked improvement of his symptoms (cough, night sweats) without being completely resolved. Disease stabilization and normalization of his general conditions lasted for 12 months.

Discussion

The following major findings emerge from this dose escalation and “proof of principle” study: (1) H22xKi-4 was well tolerated at doses up to 80 mg/m² (given on days 1, 3, 5, and 7) with only mild to moderate and transient side effects. There were no dose-limiting toxicities, and the maximum tolerated dose of this construct was not reached. (2) The mean elimination half-life of H22xKi-4 at the maximum dose given was 11.1 hours, leading to a significant accumulation of the drug as determined by Cmax and AUC by the end of one course (corresponding to 4 infusions). There was no significant change in elimination half-life, apparent volume of distribution, or clearance over the treatment period. (3) The BSM bound to the peripheral blood monocytes as well as to the malignant H-RS cells, suggesting an effective dose and schedule. (4) H22xKi-4 induced tumor responses in pts with pretreated advanced and refractory HL.

Table 3. Pharmacokinetics of H22xKi-4 (dose cohort 20 mg/m²/d, first cycle, n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Tmax, h</th>
<th>Cmax, mg/L</th>
<th>T1/2 elimination, h</th>
<th>AUCinf, L/m²</th>
<th>Vdz, L/m²</th>
<th>CI, L/h/m²</th>
<th>MRT, h</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>Mean</td>
<td>0.63</td>
<td>11.2</td>
<td>10.71</td>
<td>53.17</td>
<td>3.91</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.32</td>
<td>4.8</td>
<td>6.62</td>
<td>63.94</td>
<td>5.04</td>
<td>6.6</td>
</tr>
<tr>
<td>Day 3</td>
<td>Mean</td>
<td>0.75</td>
<td>11.5</td>
<td>15.26</td>
<td>27.56</td>
<td>2.08</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.24</td>
<td>4.3</td>
<td>10.39</td>
<td>13.04</td>
<td>1.61</td>
<td>5.9</td>
</tr>
<tr>
<td>Day 5</td>
<td>Mean</td>
<td>0.85</td>
<td>10.5</td>
<td>14.71</td>
<td>23.22</td>
<td>2.00</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.25</td>
<td>5.9</td>
<td>10.65</td>
<td>6.97</td>
<td>1.12</td>
<td>8.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>Mean</td>
<td>1.1</td>
<td>10.5</td>
<td>18.33</td>
<td>18.11</td>
<td>1.46</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.4</td>
<td>6.2</td>
<td>11.92</td>
<td>6.33</td>
<td>0.69</td>
<td>8.7</td>
</tr>
</tbody>
</table>

AUCinf indicates AUC(0-infinity).

*Median for Tmax.

Figure 1. Area under the curve of H22xKi-4 during the first course.

Figure 2. Area under the curve of H22xKi-4 during the first course.

Figure 3. Absolute number of peripheral blood monocytes and their CD64 expression during the first course.

Figure 4. Tumor responses in a patient with pleural effusion and paraplegia due to advanced HL. The toxicity profile of H22xKi-4 resembled the “cytokine-release syndrome” as described for several MoAbs against lymphoma cells, including rituximab, alemtuzumab (Campath-1H), or OKT3.35-37 These symptoms occurred at all dose levels, suggesting biologic activity even at lower doses. Only grade II fever and mild
myalgia were restricted to the highest dose level. The onset of symptoms varied but lasted no longer than 6 hours after the end of infusion. A direct correlation between side effects and the plasma concentrations of H22xKi-4 was not observed. Similar findings were recently reported by Lewis and coworkers for the MDX-H210 BsAb.38 Despite the fact that we administered comparably high doses of the BSM, we were unable to define the MTD of H22xKi-4. Six pts were treated with 80 mg/m² per course, and the highest total amount of BSM given in one patient was 740 mg. Because there were no major side effects at this dose level, 80 mg/m² per course given on days 1, 3, 5, and 7 was safe and well tolerated. In the absence of severe dose-limiting toxicities, established dose escalation schemes as used for small molecules have only very limited use. Very similar findings are known from other specific monoclonal antibodies such as rituximab. In the present study, we defined 80 mg/m² per course as biologic active dose, because we observed a saturation of the peripheral blood monocytes similar to previous studies using comparable anti-CD64 bispecific molecules.34 This, of course, is only a surrogate parameter for possible biologic activity.

The pharmacokinetic data reported in the present study have to be interpreted with care, because only 2 dose levels gave measurable BSM concentrations. In addition, there were confounding factors such as sCD30 levels, absolute monocyte counts, tumor load, and dosing intervals. The estimated mean elimination half-life of 11.1 hours is within the range reported for other anti-CD64-based BSMs.15 This half-life is shorter than that reported for other humanized IgG-based antibodies, such as rituximab, where a half-life of more than 400 hours was described. The shorter half-life of H22xKi-4 is not surprising, because this new molecule is smaller when compared with an intact IgG antibody (104 kDa versus 180 kDa) and lacks the Fc portion.25 Compared with intact antibodies, the molecular size of H22xKi-4 might more easily allow its penetration into the malignant lymph nodes.41

The rationale for the schedule used in this study was to saturate all peripheral blood monocytes and sCD30 with the BSM, resulting in an excess of unbound BSM that could then penetrate into tissues to bind H-RS cells. Binding to sCD30 might have a major impact on the distribution of H22xKi-4 and the development of side effects. Nevertheless, we observed a slight accumulation of H22x-

Ki-4 measured as plasma Cmax and AUC. When these data are taken in conjunction with the absence of any significant change over time in H22xKi-4 elimination half-life, apparent volume of distribution, or clearance, this suggests true accumulation of the drug in the body. Furthermore, sCD30 remained at very low levels after the first infusion of H22xKi-4 during the whole treatment period, probably in part due to the blockade of CD30 shedding by the Ki-4 antibody.17 In addition, we demonstrated binding of H22xKi-4 to the H-RS cells by immunohistochemistry in tumor tissue. Finally, there was a profound binding of the BSM to CD64 on the effector cells. Taken together, these results support the hypothesis of an effective dose and schedule established in this study.

Response to H22xKi-4 in this population of heavily pretreated pts with progressive disease was seen over a broad range of doses (20 mg/m² to 80 mg/m² per course). With a duration of 1 to 5 months, these remissions were not long-lasting. This might in part be due to the limited number of treatment cycles administered, which was generally confined to 2 cycles. Only 2 pts received more than 2 cycles. A longer treatment period might be useful to support the development of sustained antitumor immunity, as described for an anti-CD64-based BSM in a murine non-Hodgkin lymphoma model.42

The response to H22xKi-4 seen in this trial is in accordance with data reported from solid tumors using the anti-FcγRI MoAb H22. Induction of ADCC via binding to CD64 was demonstrated in pts with advanced breast carcinoma.43 In hormone-refractory prostate carcinoma, the anti-CD64x anti-HER2 BSM MDX-H210 showed activity even at lower doses than used in the present trial.44 The preliminary data suggest activity of H22xKi-4 as reported with other BSM-based approaches in HL. Hartmann and coworkers found efficency of an anti-CD16xCD30 bispecific antibody that was combined with interleukin-2 (IL-2) in one of these trials.22 Overall, 31 pts were treated without IL-2 costimulation in these 2 studies, and 1 CR and 3 PRs could be achieved. IL-2 costimulation resulted in another CR and PR. Targeting CD16 might be hampered by the markedly decreased natural killer cell activity in HL.66,67 In contrast, targeting CD64 and thereby recruiting monocytes as effector cells could be a more promising approach. Expression of the FcγRI receptor can be up-regulated on monocytes and
induced on neutrophils by stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF). Thus, costimulation with GM-CSF might further improve the efficacy of H22xKi-4.

In summary, 2 courses of 80 mg/m² H22xKi-4 show an excellent toxicity profile and efficacy in some pts with pretreated, advanced, or refractory HL. We plan further evaluation of this approach in a phase 2 study.

References