Phase 1 trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma

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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′) 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/m^2/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/m^2 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)

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.[1-3] 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.[4] 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.[5] The outcome is even worse for those with primary refractory disease.[6] 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.[7-11] 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.[12-15] Among the different target antigens on H-RS cells, CD30 seems to be the most promising, because it is strongly overexpressed in Hodgkin lymphoma.[16-19]

A number of different MoAbs have been evaluated for treatment of HL pts, including antibody-toxin constructs (immunotoxins), radioimmunoconjugates, and unmodified MoAbs.[20-23] 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.[24] Thus, we constructed a new bispecific molecule (BSM) based on the high-affinity FcγRI receptor. CD64 is part of this receptor and is expressed on activated neutrophils, monocytes, and macrophages.[25] 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

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to FcγRI results in increased cytotoxic activity, including cytolysis, respiratory burst, and production of oxidative enzymes.[26,27] The murine MoAb M22 binds to the FcγRI at an epitope outside the normal Fc binding domain, thereby circumventing the competition with serum IgG.[28] The binding site used for construction of the new BSM reported in this study is based on the humanized version of the anti-CD64 MoAb M22 termed H22.[29]

H22 F(ab′) fragments were chemically linked to F(ab′) fragments derived from the anti-CD30 MoAb Ki-4. The resulting bispecific molecule (BSM) H22xKi-4 has a molecular weight of 104 kDa and thus is likely to allow a better tumor penetration compared with a complete antibody. In addition, this type of construct does not activate complement or bind to noncytotoxic cells that express Fc-receptors, resulting in minimal side effects.[30] Preclinical in vitro testing of H22xKi-4 demonstrated that this BSM mediates antibody-dependent cellular cytotoxicity (ADCC) in conjunction with monocytes as well as phagocytosis in conjunction with monocyte-derived macrophages (MDM).[31] 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 amenable 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 could be administered according to the individual investigator's judgment in responding pts.

Dose escalation and major toxicity rules

MTD was defined as the highest dose level immediately below the dose at which dose-limiting toxicities occurred. This dose was defined by the occurrence of a DLT in at least 2 of 3 or 6 pts. Six pts had to be treated on the MTD. The MTD was evaluated using the accelerated titration design as follows: double-step (100%) dose escalation with one patient per cohort in the accelerated phase until the first instance of DLT at any course or the second instance of any course grade II toxicity was observed.[32] Then, the cohort of the current dose level had to be expanded to at least 3 pts, and standard modified Fibonacci dose escalation scheme (with 50% dose increments) was used for all following dose levels. Adverse events not judged to be related to the study drug were not considered as toxicity in terms of these dose escalation rules and rules for determination of the MTD. The dose groups were as follows: 1.0 mg/m^2/d, 2.5 mg/m^2/d, 5 mg/m^2/d, 10 mg/m^2/d, and 20 mg/m^2/d. A total of 6 pts were enrolled on the 20 mg/m^2/d dose level irrespective of the lack of toxicities. DLT was defined as any grade III or IV nonhematologic toxicity (according to National Cancer Institute [NCI] criteria) or grade IV hematologic toxicity excluding lymphopenia, monocytopenia, or neutropenia. Pts could start with the next dose level if the third administration of H22xKi-4 on the previous dose level had been completed without DLT. Vital signs were controlled every hour during the infusion and up to 6 hours thereafter. Pts were monitored weekly, including complete blood cell count, biochemistry, urine status, performance status, and toxicity assessment according to NCI–common toxicity criteria (CTC) criteria. After the second course (corresponding to day 28), baseline evaluations were repeated, including electrocardiograph, chest x-ray, echocardiography, lung function test, serum creatinine, and assessment of tumor response as defined below.

Drug formulation and administration

H22xKi-4 was produced using the method of Glennie as described previously.[33] The drug was supplied in sterile, 10-mL vials containing 1 mg/mL H22xKi-4 and had to be stored at 4°C. Prior to each infusion, 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 1.200*g* 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 C_{max} and T_{max} 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_e) 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 ($T_{1/2}$) was estimated from $0.693/k_e$. 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 (C_{last}/k_e). Total body clearance (CL)

was calculated by dividing the dose by $AUC_{(0-\infty)}$. The apparent volume of distribution (Vdz) was estimated from CL/k_e. The mean residence time (MRT) was estimated from AUMC/AUC. The accumulation factor R was estimated from the equation Treat 4 $AUC_{(0-\tau)}$/Treat 1 $AUC_{(0-\infty)}$. In this Treat 4, $AUC_{(0-\tau)}$ 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 H22xKi-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 H22xKi-4. This material was divided into 2 parts, one of which was immediately fresh frozen and stored at −80°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 biotylinated 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.[34] 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 4 hours after treatment (at each treatment day) using the one sample *t* test for the group of 6 pts who received the maximum dose of 20 $mg/m^2/d$.

Assessment of response

Staging was performed in accordance to the Ann Arbor classification system. Complete remission (CR) was defined as the absence of any clinical or radiologic evidence of active disease over a period of at least 4 weeks. Partial remission (PR) was defined as 50% or more decrease in the product of the 2 largest perpendicular diameters of all measurable lesions, as determined by 2 consecutive observations not less than 4 weeks apart. Less than 25% decrease or increase in total tumor mass, again persisting for at least 4 weeks, was defined as no change (NC). Progressive disease was defined as the appearance of any new lesions or an increase of more than 25% in tumor size.

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 sclerosing 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 H22xKi-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

H22xKi-4 concentrations were detectable only in those pts receiving more than 5 $mg/m^2/d$. T_{max} occurred at or after the end of the infusion in all subjects on all treatment days. The plasma H22xKi-4 concentration decay over time was monoexponential for all pts. There was a trend for C_{max} and AUC to increase over time. Therefore, a univariate, single-factor, repeated measures analysis of variance for C_{max}, $T_{1/2z}$, AUC, Vdz, and Cl over the 4-treatment 7-day period was performed on the 6 pts in the 80 mg/m^2 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 C_{max} ($F = 6.38$; $P = .005$) 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 H22xKi-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 H22xKi-4 terminal half-life was 7.9 hours at the 10 $mg/m^2/d$ dose ($n = 1$) and had a mean value of 11.1 hours at the 20 $mg/m^2/d$ 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/m^2. The mean value of the apparent volume of distribution (Vdz) in the 20 mg/m^2 group was 53.17 L/m^2. The total body clearance of H22xKi-4 on day 1 varied from 1.02 to 14.06 $L/h/m^2$, with a mean value for the group of pts who received 20 mg/m^2 of 3.91 $L/h/m^2$ (SD 5.04 $L/h/m^2$). 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.

Table 1. Patient characteristics

No.	Age, y	Sex	Histology	Stage	No. of prior chemotherapies	Time to last therapy preceding BSM, mo	No. of relapses	Dose, mg/m^2/d	No. of courses	Involvement	Response
1	26	M	MC	4B	6	2	3	1	2	Mediastinal, axillary, abdominal LN, hepatic infiltration	PD
2	22	F	MC	4B	3	7	2	2.5	2	Supraclavicular and infraclavicular, mediastinal LN, pulmonary infiltration	PD
3	42	M	NS	3A	4	3	4	5	2	Mediastinal and abdominal LN	PR, 5 mo
4	40	M	NS	4B	4	7	7	10	4	Thoracic vertebrae 5-8	PR for 4 wk, then chemotherapy for consolidation
5	40	M	MC	3B	3	18	1	20	2	Cervical, axillary, abdominal, inguinal LN, splenic infiltration	SD
6	35	M	NS	4B	4	7	4	20	3	Mediastinal LN, pulmonary plus thoracic wall infiltration	SD
7	22	F	NS	4A	2	2	1	20	2	Mediastinal LN, pulmonary and hepatic infiltration	PR for 4 wk, then MR (response of hepatic lesion, pulmonary PD)
8	53	M	NS	3A	3	5	2	20	2	Supraclavicular, axillary, mediastinal, abdominal, inguinal LN	SD
9	35	M	NS	4A	4	2	2	20	2	Pulmonary infiltration	CR, 3 mo
10	33	M	NS	4B	4	6	3	20	2	Mediastinal, axillary, abdominal LN, vertebral	SD

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).

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 2. Soluble CD30 and toxicities of H22xKi-4

Patient no.	Dose level, mg/m^2/d	sCD30 pretherapy, U/mL	sCD30 after second course, U/mL	Tachycardia, grade	Hypotension, grade	Fever, grade	Chills, grade	Myalgia, grade	Fatigue, grade	HABA*
1	1	334	321	1	1	0	0	0	1	0
2	2.5	39.6	3.7	1	1	1	0	0	1	0
3	5	145.4	6.1	0	0	1	0	0	1	0
4	10	8.8	2.3	1	0	0	1	0	1	32 768
5	20	1.7	0.1	1	1	2	1	0	1	32
6	20	87.4	1.8	0	0	0	0	0	1	64
7	20	25	Not detectable	0	0	0	0	0	1	2
8	20	3.2	5.9	1	0	2	1	1	1	64
9	20	7.7	Not detectable	0	0	0	0	1	1	8
10	20	0.1	0.1	1	1	2	1	1	1	1 024

*X-fold increase at the end of therapy compared with baseline.

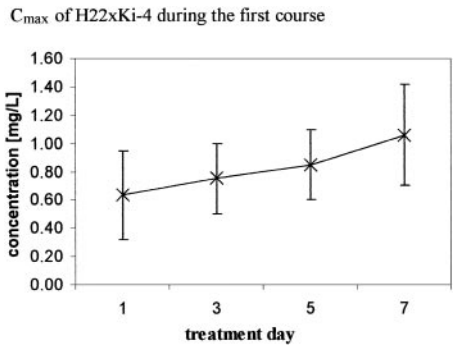

Figure 1. Maximum plasma concentrations of H22xKi-4 during the first course. C_{max} values are the means of the 6 patients treated with 20 mg/m^2/d H22xKi-4. Error bars represent the SD. There is a significant time effect on C_{max} ($P = .005$).

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] infiltration. Treatment with the BSM led to a complete resolution of neurologic defects (paraplegia) and to a measurable partial response, although B symptoms did not resolve completely. In addition, during the treatment period analgesics could be reduced. Because there was no additional improvement of the response after another 2 cycles of H22xKi-4, chemotherapy was given to minimize the risk of disease progression and a possibly fatal fracture of the sixth thoracic vertebra, which was most seriously affected.

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/m^2 (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 C_{max} 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.

H22xKi-4 is a new bispecific molecule consisting of 2 chemically linked F(ab′) fragments derived from the murine anti-CD30 MoAb Ki-4 and the humanized anti-CD64 MoAb H22. This construct had shown activity against H-RS cell lines in vitro.[31] In the present first clinical trial of H22xKi-4, the most common side effect was fatigue, which occurred in all 10 pts treated. Other side effects included tachycardia, hypotension, chills, fever, and myalgia. 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

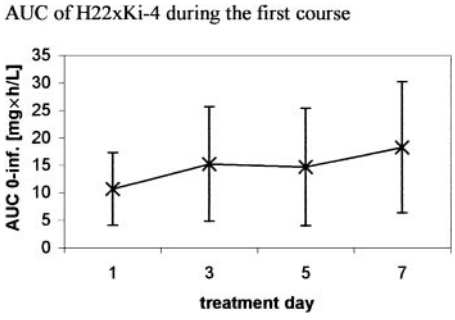

Figure 2. Area under the curve of H22xKi-4 during the first course. AUC values are the means of the 6 patients treated with 20 mg/m^2/d H22xKi-4. Error bars represent the SD. There is a significant time effect on the AUC ($P = .008$).

Table 3. Pharmacokinetics of H22xKi-4 (dose cohort 20 mg/m^2/d, first cycle, n = 6)

	T_{max}, h	C_{max}, mg/L	$T_{1/2}$ elimination, h	AUCinf, mg × h/L	Vdz, L/m^2	Cl, L/h/m^2	MRT, h
Day 1							
Mean*	4.0	0.63	11.2	10.71	53.17	3.91	16.5
SD		0.32	4.8	6.62	63.94	5.04	6.6
Day 3							
Mean*	4.0	0.75	11.5	15.26	27.56	2.08	16.8
SD		0.24	4.3	10.39	13.04	1.61	5.9
Day 5							
Mean*	4.0	0.85	10.5	14.71	23.22	2.00	15.4
SD		0.25	5.9	10.65	6.97	1.12	8.5
Day 7							
Mean*	4.0	1.1	10.5	18.33	18.11	1.46	15.3
SD		0.4	6.2	11.92	6.33	0.69	8.7

AUCinf indicates AUC(0-infinity).
*Median for T_{max}.

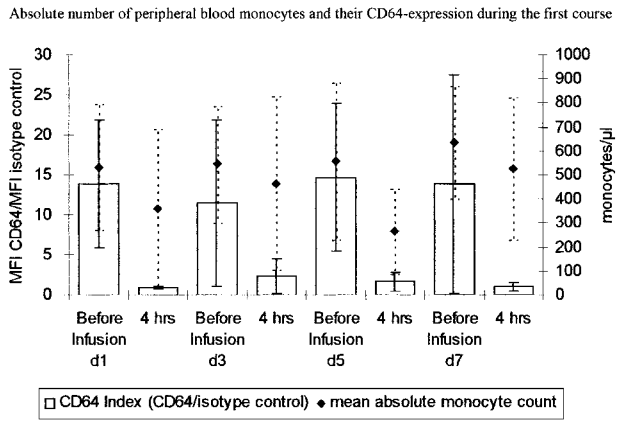

Figure 3. Absolute number of peripheral blood monocytes and their CD64 expression during the first course. CD64 expression on CD14$^+$ cells was determined by FACS analysis and correlated to an isotype control (CD64 index = CD64 mean fluorescence intensity/mean fluorescence intensity isotype control) and measured immediately before and 4 hours after the end of infusion of H22xKi-4. Values are the means of the 6 patients treated with 20 mg/m^2/d. There was a decrease of the CD64 expression on peripheral blood monocytes (day 1 $P = .025$; day 3 $P = .095$; day 5 $P = .033$; day 7 $P = .118$). Error bars represent the SD.

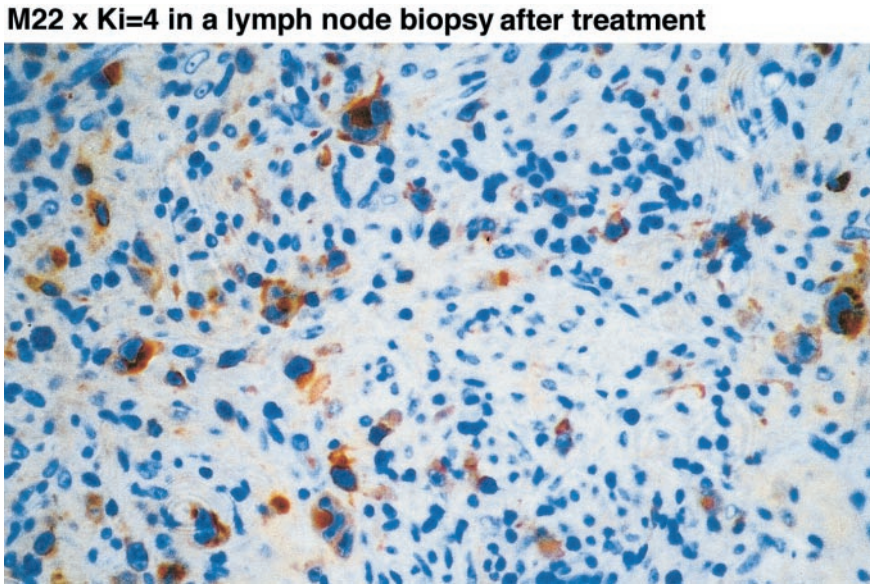

Figure 4. Immunohistochemical detection of H22xKi-4 in a lymph node biopsy after treatment. For the immunohistochemical investigation, the tissue was deparaffinized, cut into sections of 5 μm, and blocked with pig serum for 10 minutes to reduce unspecific staining. To detect the murine part of the bispecific molecule (Ki-4 F(ab′)), a polyclonal rabbit antimouse Ab was applied and incubated at 4°C overnight, followed by a biotylinated pig antirabbit antibody and a standard biotin-streptavidin kit. Finally, the slides were stained with fast red. There is clear staining of the H-RS cells. The numerous small lymphocytes in the specimen are negative. The biopsy of a second patient showed an identical staining pattern.

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/m^2 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/m^2 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.[39] In the present study, we defined 80 mg/m^2 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.[40] 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 H22xKi-4 measured as plasma C_{max} 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/m^2 to 80 mg/m^2 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 efficacy of an anti-CD16xCD30 bispecific antibody that was combined with interleukin-2 (IL-2) in one of these trials.[24,45] 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.[46,47] 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/m^2 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.

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