

A Phase I Study of an Anti-GD3 Monoclonal Antibody, KW-2871, in Patients with Metastatic Melanoma

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ABSTRACT

Purpose: KW-2871 (IgG1 kappa chimeric antibody) targets GD3, which is upregulated in melanomas. We conducted a phase I trial of KW-2871 in patients with metastatic melanoma. **Methods:** Seventeen (17) patients were enrolled and received an initial test dose (10 mg/m²) intravenously. Two (2) weeks later, patients were stratified into 4 cohorts to receive 4 doses of KW-2871 (infused over 1 hour) at 2-week intervals (20, 40, 60, and 80 mg/m²). No premedications were administered for the test or first therapeutic doses. **Results:** Dose-limiting toxicities were Grade 3 laryngospasm and chest tightness with the initial therapeutic infusion at doses of 80 and 60 mg/m². The maximum tolerated dose (MTD) was established at 40 mg/m² of KW2871. The most common side-effect was urticaria (Grades 1–3) in 16 of 16 patients during an initial therapeutic infusion without premedication. The mean terminal half-life, clearance, and area under the concentration-time curve (AUC_{0-t}) at a dose of 40 mg/m² for course 1 were 146 ± 31 hours, 28 ± 6 ml/hour, and 1922 ± 491 mcg*hour/mL, respectively. Anti-human chimeric antibody was not detected. Two (2) patients in the 40 mg/m² cohort had stable disease. **Conclusions:** An MTD of 40 mg/m² without premedication was established for KW-2871, with urticaria being the most common side-effect and dose-limiting anaphylactoid infusion reactions.

Key words: monoclonal antibody, KW-2871, melanoma, GD3

INTRODUCTION

The incidence of melanoma is increasing, with 1 of 75 Americans being diagnosed annually.^{1–3}

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Early-stage, nonmetastatic disease can be readily cured by surgical treatment⁴; however, metastatic disease is incurable with available therapeutic options.⁵ Monoclonal antibodies (mAbs) targeting melanoma-specific antigens have the potential to utilize immune effector functions to kill tumor cells. Gangliosides—glycolipids containing sialic acid—including GD3, GD2, and GM2, are highly expressed on melanoma cells, although the expression on some normal tissues is well documented.^{6–8} GD3 expression is up-regulated with malignant transformation and tu-

mor progression, and is, therefore, an attractive target for therapy.⁹

Initial clinical studies using murine anti-GD3 (R24) in patients with metastatic melanoma have demonstrated objective response rates up to 20%; however, treatment was limited by the rapid induction of human antibodies to the murine monoclonal reagents (HAMA).^{10–14} A second anti-GD3 murine mAb (KM-641) with enhanced preclinical activities^{15,16} was used to develop a chimeric construct targeting GD3 (KW-2871).¹⁷ This antibody has the variable region of the mouse mAb KM-641 and the constant region of human IgG1. Pre-clinical studies with ¹¹¹In-labeled KW-2871 showed excellent immunoreactivity and tumor localization in nude mice.^{16,17} KW-2871 mediates *in vitro* antibody-dependent cell mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of GD3 expressing cell lines and inhibits GD3 tumor xenografts in mice.^{16–18} There are no reports of anti-GD3 interaction with GD3 positive tumor cells causing inhibition of proliferation, apoptosis, or other regulatory responses.

An initial phase I dose-escalation trial examined dose cohorts of 1–40 mg/m² every 2 weeks for 3 doses.¹⁹ No Grade 3 or 4 adverse events occurred, and the maximum tolerated dose (MTD) was not established. This trial was undertaken to establish the MTD of KW-2871 when administered every other week for 4 doses.

PATIENTS AND METHODS

Study Design

This trial was an open-label, single-center, dose-escalation, phase I study to determine the MTD, safety, immunogenicity, and pharmacokinetic profile of repeated doses of KW-2871 administered intravenously (i.v.) in patients with metastatic melanoma. All patients received an initial test dose of 10 mg/m² of KW-2871 (infused over a 1-hour period). Two (2) weeks after the test dose, patients were entered into a specified dose cohort and received 4 doses given at 2-week intervals or until dose-limiting toxicity (DLT) was encountered. DLT in the protocol was defined as any Grade 3 or 4 toxicity. The protocol was initially designed with four dose cohorts (20, 40, 80, and 120 mg/m²/dose). All doses of KW-2871 were i.v. infused over 60 minutes. No premedications were allowed before the test dose or the first therapeutic infusion. Three (3) patients were

enrolled at each dose level, and dose escalation was performed if all 3 patients had no evidence of DLT. If 1 of these 3 patients had a DLT, 3 additional patients (total of 6) would be enrolled in the cohort. If 2 patients in any cohort had DLTs, the cohort will have exceeded the MTD. If the MTD is exceeded, a cohort will be added at a dose intermediate between the prior dose and the cohort which exceeded the MTD. MTD was defined as the highest dose in which less than 2 of 6 patients experienced DLT. KW-2871 was administered in the outpatient General Clinical Research Center (GCRC; University of Alabama at Birmingham, Birmingham, AL); patients were monitored for 4 hours after each infusion.

Patients were evaluated for study drug-related adverse events throughout the duration of the study and 4 weeks following the last dose of KW-2871. Evaluation for tumor response or progression²⁰ occurred 2 weeks following the fourth therapeutic dose. Patients with tumor response or stable disease at restaging were eligible to receive further doses of KW-2871, provided that they had recovered from any toxicity.

Patients

Eligible patients had pathologically proven stage IV melanoma with metastatic lesions not amenable to surgical resection and had failed standard therapy. Additional criteria included an age greater than 18 years, Eastern Cooperative Oncology Group (ECOG) performance score of 0, 1, or 2, measurable disease, and an expected survival greater than 12 weeks. No treatment with chemotherapy, radiation therapy, systemic hormonal therapy, or immunotherapy was allowed for 4 weeks before study entry. The study was reviewed and approved by the University of Alabama at Birmingham (Birmingham, AL) Institutional Review Board, and all patients understood and gave written, informed consent prior to enrollment.

Patients were excluded if they had symptomatic brain metastases or leptomeningeal disease, uncontrolled infection, known HIV infection; inadequate recovery from any prior surgical procedure, any life-threatening illness, psychiatric disorder, or altered mental status precluding understanding of the informed consent process. Patients with treated or asymptomatic brain metastases on a stable dose of corticosteroids for 4 weeks were eligible. Patients with baseline hematological abnormalities (ANC < 2000/mm³, platelet < 100,000/mm³), abnormal renal or hepatic function (total bilirubin ≥ 1.5 mg/dL; AST

or ALT $\geq 3 \times$ ULN; serum albumin ≤ 2.5 g/dL; creatinine $\geq 1.5 \times$ ULN), significant cardiovascular disease (CHF requiring therapy, anti-arrhythmic therapy, severe conduction disturbances, angina pectoris requiring therapy, or an acute myocardial infarction within 6 months) were excluded. Women who were pregnant or lactating were excluded, as well as women of childbearing potential and fertile men not agreeing to effective methods of contraception.

Investigational Agent

KW-2871, an anti-GD3 chimeric mAb, was constructed from the murine anti-GD3 KM-641 mAb, as previously described.¹⁷ A clinical-grade antibody was provided by Kyowa Pharmaceuticals, Inc. (Princeton, NJ). Vials were stored at 2–8°C under dark conditions and contained a total of 10 mL, at a concentration of 2 mg/mL. Doses were calculated based on the patient's body surface area and diluted with normal saline to a total volume of up to 250 mL (final concentration did not fall below 0.2 mg/mL). Infusions were initiated within 1 hour of preparation. The mAb was infused through a 22-micron filter.

Pharmacokinetics and Immunogenicity

Plasma specimens were collected for pharmacokinetic evaluation of course 1 (pre- and immediately postinfusion, and at 1/2, 1, 4, 24, 48, 72, 168, and 268 hours), courses 2 and 3 (prior to infusion and immediately following infusion), and course 4 (prior to infusion, immediately following infusion, and at 1/2, 1, 4, 24, 48, 72, 168, and 264 hours and day 14) at each dose. The plasma concentration of KW-2871 was measured using an enzyme-linked immunosorbent assay (ELISA), as previously described.¹⁹ The pharmacokinetic parameters for courses 1 and 4 in patients were calculated by noncompartmental analysis, using WinNonlin (version 4.0; Pharsight, Mountain View, CA). The following pharmacokinetic parameters were evaluated in patients: area under the concentration-time curve from time 0 to time t, where t was the last sampling time (AUC_{0-t}), systemic plasma clearance (CL), and the elimination half-life (T_{1/2e}).

Plasma specimens were collected prior to each dose, end of the last course, and 6 weeks after the last dose to evaluate the development of antibodies to KW-2871. The plasma concentration of anti-human chimeric antibody (HACA) was measured by ELISAs, as previ-

ously described.¹⁹ A plasma sample was considered positive for HACA if the value was equal to or ≥ 50 ng/mL.

Cytokine Response

Serum samples were collected before and immediately after the test dose in 10 patients, and before and after the first therapeutic dose in 6 patients, to assess the effects of KW-2871 on cytokines released into the peripheral blood. The specific cytokines measured included the soluble interleukin (IL)-2 receptor (sIL-2r, lower limit of detection – lld = 404 pg/mL), IL-1 a (lld = 10.2 pg/mL), IL-1b (lld = 10.2 pg/mL), IL-4 (lld = 10.2 pg/mL), IL-6 (lld = 10.2 pg/mL), IL-10 (lld = 15.3 pg/mL), and interferon-gamma (IFN- γ) (lld = 25.6 pg/mL). ELISA kits (Endogen; Woburn, MA) were used to measure the cytokine levels, according to the manufacturer's protocol, and assays were performed in triplicate.

Statistic Analysis

All variables collected in this study were summarized at the timepoint collected for each dose cohort. Continuous variables were summarized two ways: if the dose cohort had 2 patients, the exact values for both patients were listed; if the dose cohort had more than 2 patients, then the exact values were listed and the number, mean, standard error of the mean, and range were presented. Categorical variables were summarized in a similar way by listing exact values and by presenting the number and percent of patients in each category for dose cohorts with greater than 2 patients. All analyses were descriptive.

RESULTS

Patient Demographics

As shown in Table 1, a total of 17 patients (11 males and 6 females) were enrolled in the study (3 at 20 mg/m²; 6 at 40 mg/m²; 6 at 60 mg/m², and 2 at 80 mg/m²). One (1) patient in the 20 mg/m² cohort received the test dose; however, the patient was excluded from the study owing to disease progression prior to the first therapeutic dose. The mean patient age was 62.6 years (range, 36–85). Seventeen (17) patients had a previous surgical intervention, 5 had received radiation therapy, and 15 had received prior systemic ther-

Table 1. Patient Demographics

<i>Patient</i>	<i>Dose (mg/m²)</i>	<i>Age</i>	<i>PS</i>	<i>Gender</i>	<i>Therapeutic infusions (number)</i>	<i>Previous therapy</i>	<i>Previous systemic regimens</i>
1	20	59	1	M	4	Surg, ST	DTIC
2	20	76	2	M	0	Surg, ST	DTIC, IL-2, IFN
3	20	33	1	M	3	Surg, ST	DTIC, TXL, IL-2, IFN, Genesense
4	40	54	1	M	4	Rad, Surg, ST	DTIC, IFN, CDDP, VNB
5	40	60	1	F	12	Surg, ST	DTIC, IL-2, IFN
6	40	47	1	M	4	Surg, ST	DTIC, IL-2, TXL, THL
7	80	52	1	M	1	Rad, Surg	—
8	80	54	1	F	4	Rad, Surg, ST	DTIC, CDDP, VNB
9	60	68	1	F	3	Surg, ST	DTIC, IFN, TXL
10	60	44	1	F	1	Surg, ST	DTIC, IL-2
11	60	82	0	M	4	Surg	—
12	60	72	0	F	1	Surg, ST	DTIC
13	60	65	0	F	1	Surg, ST	Temodar, THL
14	60	54	2	M	1	Surg, ST	DTIC, IL-2, IFN, CDDP, VNB, TXL
15	40	69	1	M	3	Rad, Surg, ST	DTIC, IL-2, IFN, CDDP
16	40	73	1	M	2	Rad, Surg, ST	DTIC, IL-2, IFN, CDDP
17	40	68	1	M	4	Surg, ST	DTIC

PS, ECOG; ST, systemic therapy; Rad, external beam XRT; Surg, surgery; DTIC, dacarbazine; IL-2, interleukin-2; IFN, interferon; CDDP, cisplatin; VNB, vinblastine; TXL, taxol; THL, thalidomide.

apy (median of 3 regimens). No patient was taking corticosteroids at the time of the enrollment in the trial. A total of 52 therapeutic infusions were administered.

Toxicity

As can be seen in Table 2, no DLTs were seen in the 20 mg/m² cohort. Patient 1 had Grade 1 urticaria, which responded to diphenhydramine.

Table 2. Toxicity

<i>Dose</i>	<i>Patient</i>	<i>DLT</i>	<i>Toxicity</i>
20 mg/m ² (<i>n</i> = 2)	1	N	Grade 1 urticaria
	3	N	Grade 1 fever/chills, Grade 1 urticaria, Grade 2 fatigue
40 mg/m ² (<i>n</i> = 6)	4	N	Grade 2 urticaria
	5	N	Grade 2 urticaria
	6	N	Grade 1 pruritis, Grade 2 urticaria
	15	N	Grade 2 urticaria, Grade 1 fatigue, Grade 1 fever/chills
	16	N	Grade 2 urticaria, Grade 1 fever/chills
	17	N	Grade 2 urticaria, Grade 1 pruritis
80 mg/m ² (<i>n</i> = 2)	7	Y	Grade 3 urticaria, Grade 4 seizures (history of brain metastases), Grade 2 fever/chills/rigors
	8	Y	Grade 3 laryngeal edema, Grade 3 chest tightness, Grade 2 urticaria, Grade 2 hyperglycemia, Grade 1 hypomagnesemia
60 mg/m ² (<i>n</i> = 6)	9	N	Grade 2 urticaria, Grade 1 nausea/emesis, Grade 1 fever/chills
	10	Y	Grade 3 laryngeal edema, Grade 3 chest tightness, Grade 2 urticaria, Grade 1 nausea/emesis
	11	N	Grade 2 urticaria
	12	Y	Grade 3 urticaria
	13	Y	Grade 3 urticaria, Grade 1 laryngeal edema
	14	Y	Grade 3 urticaria, Grade 3 laryngeal edema, Grade 3 chest tightness

DTL, dose-limiting toxicity.

Patient 3 had the first therapeutic infusion interrupted secondary to Grade 1 fever, chills, and urticaria; these symptoms resolved with the administration of diphenhydramine and acetaminophen, and the patient completed the infusion of KW-2871. With subsequent infusions, the patient was premedicated with diphenhydramine and did not have any additional infusion reactions.

All six patients enrolled at the 40 mg/m² dose level (patients 4–6 and 15–17) had minor infusion reactions of Grade 2 urticaria with or without fever and chills (Grade 1). Patients in this cohort received a total of 29 infusions of KW-2871, with infusion reactions being most prominent on the initial therapeutic dose. Subsequent therapeutic doses were given with diphenhydramine prophylaxis with partial or complete prevention of infusion reactions. Only 2 patients had a temporary interruption of infusions. Additional toxicities in this cohort included: 1 patient with upper gastrointestinal bleeding not related to the research agent, and 1 patient with a Grade 3 elevation of liver enzymes secondary to disease progression.

Two (2) patients received KW-2871 at 80 mg/m² and both patients experienced DLT. Patient 7 had Grade 3 urticaria, Grade 4 seizures (known history of brain metastases, who previously underwent surgical resection and gamma knife therapy), and Grade 2 fever, chills, and rigors. He responded to i.v. diphenhydramine, lorazepam, and phenytoin over 2 hours and had no further sequelae. Patient 8 had Grade 3 laryngeal edema, chest tightness, and Grade 2 urticaria with the first therapeutic dose. She had complete relief of symptoms with the administration of i.v. hydrocortisone and diphenhydramine. After discussing the potential benefit or risk for further treatment with KW-2871 and other treatment alternatives, she elected to proceed with KW-2871 therapy and received 3 additional therapeutic doses without serious problems, having been pretreated with i.v. diphenhydramine, ranitidine, and dexamethasone. No patients were enrolled in the 120 mg/m² cohort, as the 80 mg/m² cohort had exceeded the MTD.

An intermediate dose cohort of 60 mg/m² was then initiated. One (1) of the first 3 patients (10) had a DLT (Grade 3 laryngeal edema and chest tightness), and all symptoms were alleviated by treatment with i.v. diphenhydramine, hydrocortisone, and phenergan; she received no further therapy. The other 2 patients (9 and 11) had Grade 2 urticaria that responded well to diphenhydramine,

and they received 3 and 4 therapeutic doses, respectively. Three (3) additional patients were entered in this cohort and all 3 had Grade 3 urticaria, with patient 13 having grade 1 laryngeal edema and patient 14 having Grade 3 laryngeal edema and chest tightness. Both patients with laryngeal edema responded promptly to i.v. diphenhydramine, hydrocortisone, and ranitidine and received no further treatment. The 60 mg/m² dose cohort thus exceeded the MTD.

Three (3) additional patients (15, 16, and 17) were added to initial 3 patients (4, 5, and 6) at the 40 mg/m² dose level, as previously described. The MTD was established at 40 mg/m².

No patient in the trial had hematologic, ophthalmic, or renal toxicity. Urticaria (Grades 1–3) was observed in 16 of 16 eligible patients on the initial therapeutic infusion and tended to decrease or not occur in subsequent infusions. The 3 cases of Grade 3 laryngeal edema and chest tightness all responded well to i.v. diphenhydramine, hydrocortisone, and ranitidine, and patient 8 went on to receive 3 additional 80 mg/m² infusions with diphenhydramine, ranitidine, and dexamethasone pretreatment. The 10 mg/m² test dose was associated with Grades 1–2 urticaria in 3 (patients 10, 13, and 14) of 17 patients who experienced DLTs at the first therapeutic dose, despite premedication with diphenhydramine. However, 3 other patients (7, 8, and 12) had no reaction to the test dose and yet experienced Grade 3 urticaria, laryngeal edema, chest tightness, or grand mal seizure with the first therapeutic infusion at either 60 or 80 mg/m².

Pharmacokinetics

The pharmacokinetics of KW-2871 on patients who completed the initial therapeutic infusion (course 1) are provided in Table 3. The mean terminal half-life, clearance, and AUC_{0–t} at a dose of 40 mg/m² were 146 ± 31 hours, 28 ± 6 mL/hour and 1922 ± 491 mcg*hour/mL, respectively.

Immunogenicity

Serial plasma assays for human antibody response to KW-2871 were all negative. This included 10 patients studied through day 42 and 8 patients who were studied beyond day 70 (15 assays determined between days 70 and 168).

Cytokine Response

Table 4 provides the cytokine serum levels prior to and following KW-2871 infusions. There were

Table 3. Pharmacokinetic Data

<i>Dose</i>	<i>Patient</i>	<i>T</i> _{1/2e} (hour)	<i>AUC</i> _{0-t} ^a (mg*hour/mL)	<i>CL</i> (mL/m ²)
20 mg/m ²	1	182	1744	18
	3	N.C.	N.C.	N.C.
40 mg/m ²	4	115	2054	33
	5	178	2771	18
	6	156	1405	33
	15	176	2044	23
	16	148	1551	27
	17	102	1706	32
80 mg/m ²	7	123	4180	32
60 mg/m ²	9	155	2899	25
	11	208	4259	14

Note. Includes all patients who received a full infusion dosage without delays.

*AUC*_{0-t}, area under the concentration-time curve; *CL*, clearance; N.C., not calculated.

^a*t* was 336 hours (14 days).

no significant increases in cytokine levels associated with the 10 mg/m² test dose, despite the fact that 3 of 10 patients had Grade 1 or 2 urticaria infusion reactions. There was a statistically significant rise in soluble IL-2 receptor levels following the therapeutic infusion of KW-2871 (paired *t* test, *p* = 0.01). These 6 patients received 40 mg/m² (*n* = 4) or 60 mg/m² (*n* = 2), and all 6 patients had Grade 2 urticaria with the infusion of KW-2871 but no Grade 3 toxicities.

Efficacy

Although efficacy was not an aim of the trial, 2 patients enrolled in the 40 mg/m² cohort had stable disease. The first patient (5) received the test dose and 12 doses of KW-2871 over 26 weeks with no

toxicity and persistent stable disease (45% reduction in tumor mass); the patient withdrew from the study owing to personal problems. The second patient (6) had stable disease after the fourth therapeutic dose of the antibody (24% reduction in tumor mass); this patient developed upper gastrointestinal bleeding secondary to a duodenal ulcer that delayed the continuation of therapy beyond the limit specified in the protocol (2-week delay) for discontinuation of KW-2871.

DISCUSSION

R24, the murine mAb to GD3, has had the most extensive clinical trial experience in melanoma owing to its antitumor efficacy.¹⁰⁻¹⁴ However, its

Table 4. Cytokine Response

<i>Cytokine</i>	<i>Test dose</i> (<i>n</i> = 10)		<i>Therapeutic dose</i> (<i>n</i> = 6)	
	<i>Preinfusion</i> (pg/mL)	<i>Postinfusion</i> (pg/mL)	<i>Preinfusion</i> (pg/mL)	<i>Postinfusion</i> (pg/mL)
Soluble IL-2 receptor	594 ± 159	610 ± 149	628 ± 139	970 ± 262
IL-1a	3.0 ± 1.4	5.3 ± 3.4	1.2 ± 1.2	2.5 ± 1.5
IL-1b	0 ± 0	0 ± 0	0 ± 0	0 ± 0
IL-4	3.8 ± 1.2	4.2 ± 1.7	4.9 ± 2.2	4.7 ± 2.2
IL-6	4.1 ± 2.3	3.6 ± 3.1	4.3 ± 2.5	6.0 ± 3.8
IL-10	18.4 ± 32.4	17.6 ± 24.3	31.2 ± 42.1	26.2 ± 31.5
IFN-γ	13.8 ± 1.2	14.2 ± 1.7	14.9 ± 2.3	14.7 ± 2.2

IL, interleukin; IFN-γ, interferon-gamma.

immunogenicity, difficulty with radiolabeling, and serious infusion-related toxicity led to the development of alternate anti-GD3 reagents. One of these reagents, KM-641,¹⁵ was reported to differ from R24 in that it had enhanced GD3 binding to melanoma tumor cell lines and GD3 specificity to a different sialic acid motif.¹⁵ A chimeric mouse-human construct (KW-2871) of this antibody had improved *in vitro* cytotoxicity to melanoma tumor cells and enhanced antitumor efficacy in xenogeneic nude mouse models, as compared to the murine reagents.¹⁷ This reagent radiolabeled with ¹¹¹In had excellent melanoma tumor localization in mouse models¹⁶ and a human trial.¹⁹

The initial phase I trial of KW-2871¹⁹ utilized 3 infusions at 2-week intervals at dose levels of 1–40 mg/m² without reaching the MTD (no Grade 3 or 4 toxicity was observed). Patients had urticaria, fever, and rhinitis at doses $\geq$ 10 mg/m² and 1 patient had an objective partial response lasting 11 months. The terminal half-life of KW-2871 was reported as 7.68 ± 2.94 days.

This report describes our attempt to reach higher dose levels of KW-2871 given in 4 infusions at 2-week intervals and to describe the toxicity profile at higher dose levels. We also introduced an initial “test dose” of 10 mg/m² to screen for patient sensitivity to the reagent and/or possibly “desensitize” the patient’s response to subsequent higher doses. Because of the lack of Grade 3 or 4 toxicity in the initial phase I trial,¹⁹ the schedule selected for this trial was a 1-hour infusion of the mAb every 2 weeks.

We found that the dose cohort of 40 mg/m² $\times$ 4 (total dose of 170 mg/m² over 9 weeks) was well tolerated, whereas dose levels of 80 or 60 mg/m² produced Grade 3 “anaphylactoid” infusion reactions, which were dose-limiting and exceeded the MTD. The “test dose” did not appear to reduce reactions for subsequent therapeutic doses, as 16 of 16 initial therapeutic infusions were associated with urticaria at all dose levels. A positive test dose appeared to be correlated with the occurrences of DLT reactions, whereas a negative test dose did not predict which patients would have DLTs. The skin toxicity and systemic reactions were similar, but not identical, to the toxicity profile of other GD3 monoclonal reagents.^{10–14} The systemic infusion reactions owing to R24 were reported to be resulting, in part, to the antibodies’ cross-reactivity with a subpopulation of lymphocytes in the peripheral blood^{21,22} and in melanoma metastases.²³

R24 has shown, in previous preclinical evaluations, its ability to increase IL-2 receptor expression, cell proliferation, and cytokine release from T-cells *in vitro*.^{21,22} We have analyzed the effect of KW-2871 on *in vitro* lymphocyte proliferation and cytokine release. As compared to R24, KW-2871 produced no lymphocyte proliferation and minimal changes in lymphokine expression, using cells from normal donors.²⁴ Our attempt at identification of lymphokine release with KW2871 infusions *in vivo* were unsuccessful, although we did not examine serum samples from patients with DLTs. However, the significant elevation of soluble IL-2 receptor levels in post-therapeutic infusions, and not after “test dose” infusions, suggests a dose-dependent T-lymphocyte-antibody interaction. The lack of *in vitro* or *in vivo* evidence of KW-2871-mediated lymphokine response suggests that the anaphylactoid dose-limiting toxicities may be a result of antibody mast-cell interactions.²⁵

The KW-2871 plasma elimination half-life of 146 ± 31 hours (6.1 ± 1.3 days) was similar to the prior phase I report of 7.68 ± 2.94 days on the radiolabeled bifunctional chelator conjugate of the antibody and 9.61 ± 4.16 days based on an ELISA assay of the KW-2871.¹⁹ Similarly, neither KW-2871 trial reported human antibody response to the chimeric antibody.

CONCLUSIONS

The amelioration of “anaphylactoid” reactions with hydrocortisone, diphenhydramine, and ranitidine, as well as patient number 8’s ability to tolerate 3 additional infusions of 80 mg/m² with premedication, despite an initial “anaphylactoid” reaction, was promising. This formed the basis of a new phase I trial of KW-2871, using a rigorous prophylactic drug regimen (corticosteroids, diphenhydramine, leukotriene receptor antagonist, and ranitidine) with increasing doses of KW-2871. An alternative strategy may be to limit infusion rates to no more than 20 mg/m²/hour, as this dose administered over 1 hour was well tolerated.

REFERENCES

1. SEER Cancer Statistics Review, 1975–2002. Online document at: <http://seer.cancer.gov> (Last accessed July, 2006).
2. Desmond RA, Soong SJ. Epidemiology of malignant melanoma. *Surg Clin North Am* 2003;83:1.

3. Balch CM, Buzaid AC, Soong SJ, et al. New TNM melanoma staging system: Linking biology and natural history to clinical outcomes. *Semin Surg Oncol* 2003;21:43.
4. Essner R. Surgical treatment of malignant melanoma. *Surg Clin North Am* 2003;83:109.
5. Lens MB, Eisen TG. Systemic chemotherapy in the treatment of malignant melanoma. *Exp Opin Pharmacother* 2003;4:2205.
6. Ritter G, Livingston PO. Ganglioside antigens expressed by human cancer cells. *Semin Cancer Biol* 1991;2:401.
7. Pukel CS, Lloyd KO, Travassos LR, et al. GD3, a prominent ganglioside of human melanoma: Detection and characterization by mouse monoclonal antibody. *J Exp Med* 1982;155:1133.
8. Dippold WG, Lloyd KO, Li LT, et al. Cell-surface antigens of human malignant melanoma: Definition of six antigenic systems with mouse monoclonal antibodies. *Proc Natl Acad Sci USA* 1980;77:6114.
9. Cheresch DA, Harper JR, Schulz G, et al. Localization of the gangliosides GD2 and GD3 in adhesion plaques and on the surface of human melanoma cells. *Proc Natl Acad Sci USA* 1984;81:5767.
10. Kirkwood JM, Mascari RA, Edington HD, et al. Analysis of therapeutic and immunologic effects of R24 anti-GD3 monoclonal antibody in 37 patients with metastatic melanoma. *Cancer* 2000;88:2693.
11. Houghton AN, Mintzer D, Cordon-Cardo C, et al. Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: A phase I trial in patients with malignant melanoma. *Proc Natl Acad Sci USA* 1985;82:1242.
12. Caulfield MJ, Barna B, Murthy S, et al. Phase Ia-Ib trial of an anti-GD3 monoclonal antibody in combination with interferon-alpha in patients with malignant melanoma. *J Biol Response Mod* 1990;9:319.
13. Vadhan-Raj S, Cordon-Cardo C, Carswell E, et al. Phase I trial of a mouse monoclonal antibody against GD3 ganglioside in patients with melanoma: Induction of inflammatory responses at tumor sites. *J Clin Oncol* 1988;6:1636.
14. Bajorin DF, Chapman PB, Wong G, et al. Phase I evaluation of a combination of monoclonal antibody R24 and interleukin 2 in patients with metastatic melanoma. *Cancer Res* 1990;50:7490.
15. Ohta S, Honda A, Tokutake Y, et al. Antitumor effects of a novel monoclonal antibody with high binding affinity to ganglioside GD3. *Cancer Immunol Immunother* 1993;36:260.
16. Lee FT, Rigopoulos A, Hall C, et al. Specific localization, gamma camera imaging, and intracellular trafficking of radiolabeled chimeric anti-GD3 ganglioside monoclonal antibody KM871 in SK-MEL-28 melanoma xenografts. *Cancer Res* 2001;61:4474.
17. Shitara K, Kuwana Y, Nakamura K, et al. A mouse/human chimeric anti-(ganglioside GD3) antibody with enhanced antitumor activities. *Cancer Immunol Immunother* 1993;36:373.
18. Hanai N, Nakamura K, Shitara K. Recombinant antibodies against ganglioside expressed on tumor cells. *Cancer Chemother Pharmacol* 2000;46(Suppl.):S13.
19. Scott AM, Lee FT, Hopkins W, et al. Specific Targeting, biodistribution, and lack of immunogenicity of chimeric anti-GD3 monoclonal antibody KM871 in patients with metastatic melanoma: Results of a phase I trial. *J Clin Oncol* 2001;19:3976.
20. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205.
21. Welte K, Miller G, Chapman PB, et al. Stimulation of T-lymphocyte proliferation by monoclonal antibodies against GD3 ganglioside. *J Immunol* 1987;139:1763.
22. Norihisa Y, McVicar DW, Ghosh P, et al. Increased proliferation, cytotoxicity, and gene expression after stimulation of human peripheral blood T-lymphocytes through a surface ganglioside (GD3). *J Immunol* 1994;152:485.
23. Hersey P, Jamal O. Expression of the gangliosides GD3 and GD2 on lymphocytes in tissue sections of melanoma. *Pathology* 1989;21:51.
24. Triozzi PL, Shah JJ, Wang WQ, et al. Anti-GD3 monoclonal antibody effects on lymphocytes and antibody-dependent cellular Cytotoxicity (ADCC). *Cancer Biother Radiopharm* 2006;21:555.
25. Kovarova M, Rivera J. A molecular understanding of mast cell activation and the promise of antiallergic therapeutics. *Curr Med Chem* 2004;11(15):2083.