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Pharmacokinetics of anti-ganglioside GD2 mAb 14G2a in a phase I trial in pediatric cancer patients

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Abstract A phase I trial of a murine anti-ganglioside (GD2) monoclonal antibody (mAb) 14G2a was conducted in 14 neuroblastoma patients and 1 osteosarcoma patient to assess its safety, toxicity and pharmacokinetics in pediatric patients. The pharmacokinetics of mAb 14G2a were biphasic with a $t^{\alpha}_{1/2}$ of 2.8 ± 2.8 h and a $t^{\beta}_{1/2}$ of 18.3 ± 11.8 h. In general, $t^{\beta}_{1/2}$ was dose-dependent with a level of significance of $P = 0.036$, and it reached a plateau at doses of 250 mg/m^2 or more. Overall the peak serum levels were dose-dependent at $P < 0.001$. However, they demonstrated an abrupt increase between doses of 100 mg/m^2 and 250 mg/m^2. The latter two suggest a saturable mechanism for mAb elimination. In addition, peak serum concentrations were observed earlier at higher mAb doses, which indicates the achievement of a steady state. The $t^{\beta}_{1/2}$ of mAb 14G2a in children appears to be shorter than in adults. Furthermore, 2 patients demonstrated a considerable decrease in $t^{\beta}_{1/2}$ following retreatment with 14G2a. This was paralleled by high human anti-(mouse Ig) antibody levels. This study represents the first comprehensive analysis of murine mAb pharmacokinetics in children and will be useful in the future design of mAb therapy.

Key words mAb 14G2a · Pharmacokinetics · Neuroblastoma · Ganglioside GD2 · Pediatric patients

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Introduction

Ever since the development of hybridoma technology by Köhler and Milstein, numerous monoclonal antibodies (mAb) have been generated against various tumor-associated antigens [3, 7, 9, 17, 22, 33]. One of the major promises of this new technology was that eventually such mAb could be used for tumor-targeted cancer therapy [23]. For the last decade, this novel approach has been under active investigation in multiple clinical trials, especially for use in radioimmunoimaging and radioimmunotherapy [12, 19, 27, 36]. The overall clinical efficacy obtained thus far in end-stage cancer patients was either inconclusive or relatively poor.

However, some mAb themselves demonstrated antitumor efficacy in vivo. Cheung et al. reported clinical efficacy of a murine anti-disialoganglioside (anti-GD2) mAb 3F8 in some patients with neuroblastoma and melanoma [3, 5]. Using another murine anti-GD2 mAb 14.18 and its isotypic variants, Mujoo et al. showed that these mAb suppressed the growth of xenografted tumors of neuroectodermal origin in athymic *nu/nu* mice [30]. The tumor-suppressive effects of these mAb correlate with their biological functions such as complement-dependent cytotoxicity and antibody-dependent cytotoxicity and rank in the following order:

14G2a = 14.18 > 14G2b > 14G1 [31]. On the basis of these findings, we as well as Handgretinger et al. initiated phase I clinical trials of mAb 14G2a. The clinical efficacy of 14G2a in the treatment of recurrent neuroblastoma has been reported previously [16, 18]. We report here the pharmacokinetic analysis of mAb 14G2a in our phase I trial in pediatric patients.

Materials and methods

mAb 14G2a

Anti-GD2 mAb 14G2a is an IgG2a isotype switch variant of mAb 14.18 (IgG3), obtained from the supernatant of the murine hybridoma cell line 14G2a [31]. The antibody was generated in mass culture and supplied by Biotechnetics Inc. (San Diego, Calif.). It was free of murine viruses, bacteria, fungi, *Mycoplasma* and pyrogens. This study was conducted under Dr. Yu's IND No. 2900, in accordance with the requirements of the Helsinki Declaration of 1975 and with the approval of the Institutional Review Board and the Clinical Research Center Advisory Board of the University of California at San Diego.

Patient eligibility

Patients participating in this trial had to be diagnosed as having either neuroblastoma or osteosarcoma, and to have presented with measurable or evaluable disease, which persisted or recurred after at least one regimen of therapy. The patients' minimum life expectancy had to be at least 1 month. They were required to have been off therapy for at least 4 weeks prior to antibody treatment, unless they showed signs of progression. Patients had to have recovered from toxicities of previous therapy. A reasonable performance status with a Karnofsky score greater than 60%, and adequate renal and hepatic function with serum creatinine and bilirubin levels 1.5 times the normal level or lower were required. Informed written consent was obtained from the parents of all patients entering this protocol.

Clinical trial protocol and patient characteristics

A group of 15 patients with refractory neuroblastoma and osteosarcoma were treated with five different dose levels of mAb 14G2a. In the absence of dose-limiting toxicities, the mAb dose was escalated in successive cohorts of 3 patients as follows: 25 mg/m^2, 50 mg/m^2, 100 mg/m^2, 250 mg/m^2 and 500 mg/m^2. mAb 14G2a was administered intravenously. The total antibody amount scheduled was split into five single doses and administered over 5 days in daily 5-h infusions.

Twelve boys and 2 girls, between 3 and 15 years of age and diagnosed with stage D neuroblastoma, received 16 courses of murine mAb 14G2a. In addition, a 15.5-year-old female patient (patient 15) with refractory osteosarcoma, which tested positive for GD2 by immunohistochemical staining, was included in this study. For pharmacokinetic analysis patients were divided into five dose groups of 25 mg/m^2, 50 mg/m^2, 100 mg/m^2, 250 mg/m^2 and 500 mg/m^2. Patient 5, who was scheduled to receive a dose of 100 mg/m^2 14G2a, received only 80 mg/m^2 because of the rapid progression of his disease. He was included in the 100 mg/m^2 dose group. Patient 15, who was scheduled to receive 500 mg/m^2, because of a temporary shortage of murine 14G2a only received 352 mg/m^2 14G2a. She was included in the 500 mg/m^2 dose group. Two patients, 2 and 9, received two consecutive treatments with mAb 14G2a at dose levels of 25 mg/m^2 and 50 mg/m^2 (patient 2) and 250 mg/m^2 and 500 mg/m^2 (patient 9). The time interval between the two treatments was 144 days for patient 2 and 112 days for patient 9 (Table 1).

Table 1 Treatment and dose schedule for a phase I trial of murine mAb 14G2a

Dose (mg/m^2)	Patient 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
25	X	X	X												
50		X[a]		X											
80					X[c]										
					↓										
100					X	X	X								
250								X	X	X	X				
352															X[c]
															↓
500									X[b]			X	X	X	X

[a] Patient 2 received two consecutive treatments at an interval of 144 days
[b] Patient 9 received two consecutive treatments at an interval of 112 days
[c] Patients 5 and 15 were included in the 100 mg/m^2 and 500 mg/m^2 dose group respectively

The age distribution in our overall patient population showed a mean of 7.2 ± 4.2 years and a median of 6 years with a range of 3–15.5 years. Among different dose groups there is also considerable variation in age distribution. Age means scattered between 4.9 ± 1.4 years in the 100-mg/m^2 dose group and 8.1 ± 5.6 years in the 250-mg/m^2 group (Table 2).

Blood sampling

Blood samples were collected for the determination of 14G2a levels prior to the start of antibody infusion and at the end of the first infusion on treatment day 1 (EOI 1) and 1, 2, 4, 8 and 12 h thereafter. Blood was then drawn prior to and at the end of each infusion on days 2, 3 and 4. Following the fifth and final 14G2a infusion, samples were drawn at EOI 5 and 1, 2, 4, 8, 12, 16, 20 and 24 h thereafter, then daily for up to 5 days following the final infusion. In some patients, sampling was performed at various times for up to 24 days after the final mAb administration.

Measurement of serum 14G2a levels

The serum level of mAb 14G2a was assayed by a competitive radioimmunoassay by the double-antibody technique described by Midgley, and modified by LoBuglio et al. [25, 29]. Briefly, 14G2a was labeled by the iodogen method [39]. Rabbit antisera to 14G2a F(ab')$_2$, produced by repeated immunization of white New Zealand rabbits, were a generous gift of Dr. M. B. Khazaeli, University of Alabama, Birmingham [21]. A 1:5000 dilution of this antibody produced maximum binding of ^{125}I-14G2a and was used in this assay. A standard curve of 14G2a, ranging from 50 ng to 100 μg was obtained by diluting 14G2a in normal human serum. Patient serum diluted appropriately with human serum was incubated with rabbit anti-[mouse 14G2a F(ab')$_2$] and ^{125}I-14G2a (approximately 100 000 cpm). Three serial dilutions of each patient's serum sample were assayed in duplicate. After 16–24 h of incubation at 4 °C, goat anti-(rabbit IgG) (American Qualex, La Mirada, Calif) was added to each tube at an appropriate dilution in phosphate-buffered saline containing 1% polyethylene-glycol. The bound ^{125}I-14G2a was precipitated by centrifugation at 4 °C and the radioactivity determined with a Beckman gamma counter. Only 14G2a concentrations

Table 2 α and β half-lives of mAb 14G2a in 15 pediatric patients with GD2-positive malignancies

Patient	Age (years)	Patient	mAb dose	α half-life (h)		β half-life (h)	AUC β (μg h) ml^{-1}	Treatment courses
				Day 1	Day 5			
M.Si.	3.0	1	25	4.0	0.2	7.9	75.3	1
K.K	7.5	2	25	0.5	0.7	22.4	140.9	1
		2	50	0.1	0.1	10.4	146.3	2
B.B.	12.0	3	25	0.9	0.9	3.8	54.2	1
K.O.	5.0	4	50	3.2	[b]	12.8	105.7	1
R.P.	4.2	5	80	0.9	7.7	14.1	264.5	1
T.T.	6.5	6	100	2.4	0.7	11.0	280.9	1
E.C.	4.0	7	100	0.2	1.0	4.6	12.6	1
J.B.	4.0	8	250	1.9	3.7	7.3	490.9	1
M.H.	3.0	9	250	0.9	0.9	36.5	2657.9	1
		9	500	1.6	[b]	18.2	1061.9	2
M.Se.	10.2	10	250	2.1	3.3	16.1	650.4	1
B.K.	15.0	11	250	6.4	21.3	41.8	1011.8	1
M.D.	6.0	12	500	4.5	[b]	14.8	2758.8	1
A.M.	7.5	13	500	5.6	4.6×10^{-5}	23.9	2278.2	1
J.S.	3.9	14	500	10.8	17.1	26.7	2445.7	1
L..L[a]	15.5	15	352	1.8	1.4	38.5	4307.1	1

[a] Osteosarcoma patient
[b] Could not be determined because of total overlap with $t^{\beta}_{1/2}$

measured within the linear part of the standard curve were used for determination of pharmacokinetics. Samples not meeting this criterion had to be re-assessed at different serum dilutions. The assay had a sensitivity of less than 50 ng/ml, an intra-assay coefficient of variation below 5% and an interassay coefficient variation below 5%.

Data analysis and statistical methods

The time-dependent decrease of 14G2a serum concentrations best fit a two-compartment model, revealing pharmacokinetically different slopes for the α and β half-lives [26]. The kinetics of 14G2a serum levels was assessed according to the following equation: $c = Ae^{-K_1 t} + Be^{-K_2 t}$ [26]. K_1 and K_2, the time/elimination constants, were determined by non-linear regression analysis. The half-life was determined according to: $t^{\alpha}_{1/2} = \ln 2/K_1$ and $t^{\beta}_{1/2} = \ln 2/K_2$ [8]. The area under the curve (AUC) was determined by using a computer program (PC!Info, version 3.0) and equals the integral of the 14G2a-elimination curve. Statistical significance was evaluated by Student's *t*-test as well as by linear and multiple regression analysis, whenever appropriate.

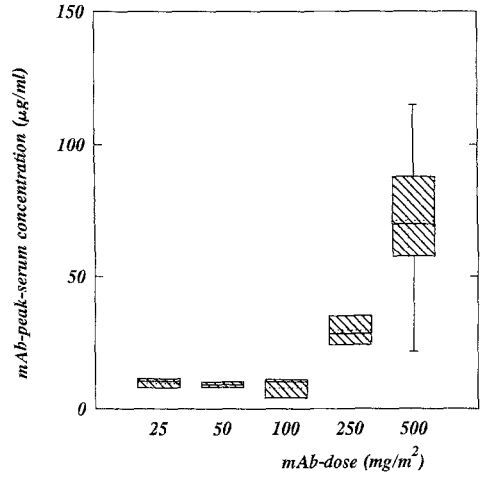

Fig. 1 Box-plot analysis of peak serum levels of mAb 14G2a compared to mAb doses administered

Results

Peak serum levels

Overall, the peak serum concentration of mAb 14G2a increased with mAb dose administered ($P < 0.001$; $r = 0.804$). However, there is no predictable increase in peak serum levels for doses of 25–100 mg/m^2. The correlation was not significant ($r = -0.371$, $P = 0.366$). At higher doses of 100–500 mg/m^2, abruptly increasing peak serum levels parallel the increasing mAb doses administered ($r = 0.752$, $P = 0.005$) (Fig. 1, Table 3). Surprisingly, serum levels of mAb 14G2a in many patients failed to show a steady increase over the entire 5-day administration period. In other words, the highest peak serum level was not detected at the end of the final infusion as has been observed for many chemical drugs [1, 8]. Instead, 5/9 patients treated with mAb 14G2a doses of at least 250 mg/m^2 reached peak serum levels of mAb 14G2a at the end of the third daily infusion, while 3/9 patients showed peak antibody levels at the end of their fourth infusion and another patient 1 h after his first infusion. In contrast, 5/8 patients treated with less than 250 mg/m^2 14G2a showed peak serum mAb concentrations at the end of their last infusion, whereas the remaining 3 patients reached peak serum levels at EOI 2, EOI 3 and EOI 4.

Table 3 Peak serum levels of mAb 14G2a at different treatment doses. Correlations between peak serum levels and mAb doses infused were $P < 0.001$, $r = 0.804$

Dose (mg/m²)	n	$X \pm$ SD (μg/ml)	Median (μg/ml)	Range (μg/ml)
25	3	9.7 ± 2.4	10.4	7.0– 11.6
50	2	9.4 ± 2.6	9.4	8.0– 10.6
80/100	3	7.8 ± 5.0	10.1	2.0– 11.3
250	4	29.5 ± 8.6	28.2	20.5– 41.2
352/500	5	71.0 ± 33.4	69.8	21.4–115.2

Pharmacokinetics

Pharmacokinetics of 14G2a *in the overall patient population*

The mAb-serum clearance was assessed during the first 24 h after administration of the first mAb dose on treatment day 1, as well as following the fifth mAb infusion. The kinetics of serum mAb 14G2a levels in our patients best fit a two-compartment model. The α half-life did not show much variation following the first mAb infusion (EOI 1) and the last mAb infusion (EOI 5). Thus $t^{\alpha}_{1/2}$, the distribution half-life, ranged from 0.1 h to 10.8 h with a mean of 2.8 ± 2.8 h and a median of 1.9 h, as assessed after the first mAb infusion. The elimination half-life, $t^{\beta}_{1/2}$, was determined after the final infusion and showed a mean of 18.3 ± 11.8 h and a median of 14.8 h with a range of 3.8–41.8 h. Despite a wide range of variation in $t^{\alpha}_{1/2}$ and $t^{\beta}_{1/2}$, statistical analysis indicates that they are significantly different from each other at $P < 0.05$. This finding confirms the two-compartment kinetics observed (Table 2).

Pharmacokinetics of 14G2a *upon repetitive mAb applications*

Patient 2 and patient 9 received two courses of 14G2a. Both showed a considerable decrease in $t^{\beta}_{1/2}$ after their second treatment as compared to their first treatment. Thus, patient 2 had a $t^{\beta}_{1/2}$ of 22.4 h following his first mAb therapy and a $t^{\beta}_{1/2}$ of 10.4 h after his second treatment. Likewise patient 9 showed a decrease in $t^{\beta}_{1/2}$ from 36.5 h to 18.2 h for his first and second courses of mAb administration respectively (Fig. 2a, b).

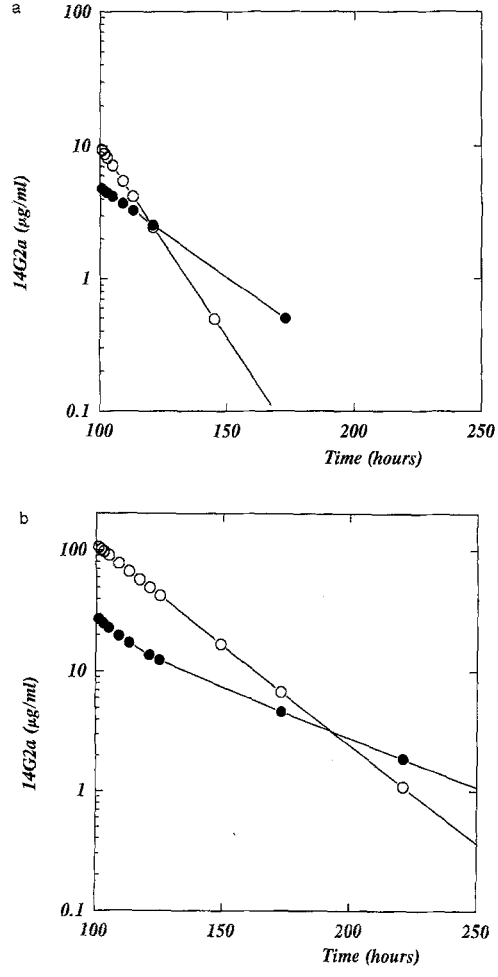

Fig. 2a, b Comparison of elimination kinetics of mAb 14G2a between the first and second course of treatment. **a** Patient 2 received 25 mg/m² mAb 14G2a in the first course (●), and 50 mg/m² in the second course of treatment (○). **b** Patient 9 received 250 mg/m² mAb 14G2a in the first course (●), and 500 mg/m² in the second course of treatment (○)

Pharmacokinetics in relation to mAb dose and patient age

When patients were grouped according to mAb treatment doses, the overall half-life variance within the group was reduced (Fig. 3a) and the median and mean β half-life for each dose group increased with higher dose levels.

Linear regression analysis of a possible correlation between $t^{\alpha}_{1/2}$, $t^{\beta}_{1/2}$ and mAb dose revealed three interesting findings. (a) Analysis of the entire patient population revealed a positive correlation between $t^{\beta}_{1/2}$ and the mAb dose administered with a P-value of 0.036 and a correlation coefficient (r) of 0.512 for a total of 17 treatment course administered. (b) Similarly, $t^{\alpha}_{1/2}$ showed a significant positive correlation with the mAb dose infused, at $P = 0.016$ and $r = 0.563$ (Fig. 3b). (c) Analysis of the median β half-life for each dose group further strengthened the above notion. Thus a positive correlation was detectable between the

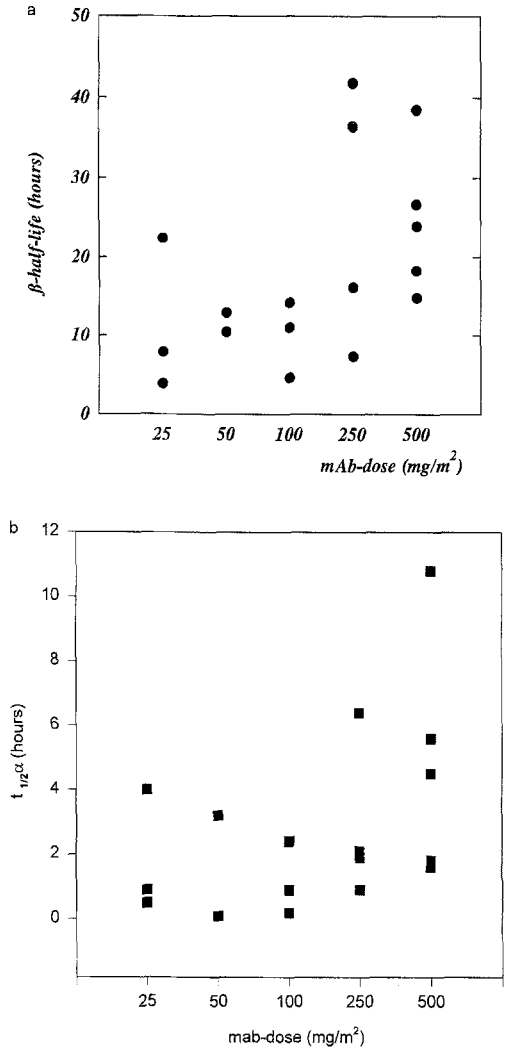

Fig. 3a, b Relationship between α and β half-life and mAb dose administered. **a** The β half-life for each treatment course is presented according to mAb dose groups. **b** The α half-life for each treatment course is presented according to mAb dose groups

median $t^{\beta}_{1/2}$ and mAb dose with $r = 0.835$ ($P = 0.079$) or $r = 0.857$ ($P = 0.063$) for all 17 treatment cycles or 15 primary cycles respectively.

Multiregression analysis of the combination of mAb dose and age indicated their correlation with $t^{\beta}_{1/2}$ ($P = 0.03$; $r = 0.628$).

Similarly, although the P-value and correlation coefficient of peak serum levels and $t^{\beta}_{1/2}$ alone did not suggest a correlation between them ($P = 0.111$; $r = 0.401$), the addition of age as a third parameter resulted in a P value of 0.054 and an r value of 0.585. This suggests considerable influence of a combination of both peak serum levels and age on $t^{\beta}_{1/2}$.

Influence of β half-life and mAb-dose on AUC

Actual drug exposure, as reflected by AUC (Table 2) shows a significant correlation with $t^{\beta}_{1/2}$ as indicated by $P = 0.002$ and $r = 0.699$. Linear regression analysis of the entire patient population indicated a clear dependence of AUC on 14G2a doses administered. P was less than 0.001 with $r = 0.743$, which indicates a high correlation of AUC with mAb dose. We further examined the influence of a combination of both mAb dose and $t^{\beta}_{1/2}$ on drug exposure by multi-regression analysis. $P < 0.0005$ and $r = 0.847$ indicated a strong impact of both factors on AUC values.

Discussion

Although a number of phase I and II clinical trials of murine mAb have been performed in adults thus far [3, 7, 9, 12, 17, 23, 27, 33], only a few of such trials were conducted in pediatric patients with solid tumors, and comprehensive analyses of mAb pharmacokinetics in children are therefore not available [3, 5, 16, 32, 34].

Pizer et al. reported some pharmacokinetic data, obtained following intrathecal administration of ^{131}I-labeled mAb HD37 and WCMH 15.14 to 6 children with CNS leukemia. The systemic antibody half-life ranged from 14.5 h to 55 h and showed a one-compartment clearance, whereas the cerebrospinal fluid clearance profile in 2–3/6 patients revealed two-compartment kinetics with a $t^{\alpha}_{1/2}$ of 2.5–3 h and β half-lives of 14.5 h and 25.75 h, respectively [34].

In the three recent clinical trials of murine anti-GD2 mAb in children with advanced neuroblastoma, relatively little information on mAb distribution and elimination was provided. In their reports on a phase I study and a phase II trial of a murine IgG3 anti-GD2 mAb 3F8, Cheung et al. concentrated on presenting clinical data. Pharmacokinetic data were not determined [3–5, 40]. In a study of anti-GD2 mAb 14G2a Handgretinger et al. reported pharmacokinetic data from 3/9 patients treated, with α half-lives ranging from 0.66 h to 1.98 h and β half-lives from 30.13 h to 53.33 h [16]. In another phase I trial of mAb 14G2a, with a very heterogeneous group of patients, including 6 pediatric patients with neuroblastoma and 17 adult patients, Murray et al. observed a wide variation of elimination half-lives ranging from 7 h to 162 h [32]. All patients presented with a variety of GD2-positive malignancies [32]. The pharmacokinetics in the latter two trials, as well as cerebrospinal-fluid clearance data presented by Pizer et al., showed biphasic mAb clearance consistent with our findings reported here.

However, in comparison to the above studies, our values for $t^{\alpha}_{1/2}$ and $t^{\beta}_{1/2}$ displayed a greater range of variation from 0.1 h to 10.8 h for $t^{\alpha}_{1/2}$ and from 3.8 h to 41.8 h for $t^{\beta}_{1/2}$. While the mean $t^{\alpha}_{1/2}$ of 2.8 ± 2.8 h is comparable to the α half-lives reported by Handgretinger et al. and Murray et al., our mean $t^{\beta}_{1/2}$ was shorter than that reported by the latter two investigators [16, 32]. Different methodologies used for the measurement of 14G2a serum levels may in part account for the

discrepancy. In addition, the impact of age on the metabolism of chemical compounds has been well established in pediatric pharmacology [2]. Faster drug clearance in children has been ascribed to increased hepatic metabolism and renal clearance during childhood. Consistent with this notion is our finding of a weak correlation between the $t^{\beta}_{1/2}$ of 14G2a and age in the present study. Aside from age, mAb dose may also affect the plasma clearance of 14G2a, as shown by a positive correlation between the mAb dose infused and $t^{\beta}_{1/2}$ ($P = 0.036$; $r = 0.512$) observed in our study. The latter is supported by Handgretinger et al., who reported shorter β half-lives at lower mAb doses in the three cases examined [16].

Previous reports by Goodman et al. [13] and Frodin et al. [10] indicate an overall positive correlation between antibody dose and antibody half-life. In addition, Haisma et al. demonstrated slower urinary excretion of mAb 16.88 at higher doses [15]. Zalutsky et al., however, reported the opposite finding, namely a decreasing blood half-life at increasing antibody doses [41]. The reason for this discrepancy is unclear and has been discussed earlier by Zalutsky et al. [41]. Three differences in the study design may be relevant. First the route of mAb administration: in contrast to the first of the groups mentioned above, Zalutsky et al. administered an anti-tenascin mAb via the intracarotid route. Secondly the target antigen: the expression of tenascin in normal liver cells and spleen red pulp sinusoids may have influenced the pharmacokinetics reported [41]. Thirdly tumor localisation: the patients treated in the first studies suffered from extracranial tumors [10, 13, 15], whereas Zalutsky et al. treated malignant gliomas [41]. It should be pointed out, however, that the impact of these differences in study design and patient population on the pharmacokinetics/dose relation of mAb remains speculative.

The use of anti-GD2 mAb is not restricted to pediatric tumors. So far, two phase I studies of 14G2a in adults with GD2-positive tumors have been conducted [32, 35]. Pharmacokinetics of mAb 14G2a were reported for both trials and in neither trial was mAb elimination dose-dependent [21, 32, 35]. Saleh et al. and Khazaeli et al. [21, 35] as well as Murray et al. [32] used 0.5 mg [21, 35] and 20 mg [32] radiolabeled ^{131}I-14G2a, which was infused together with the first dose of unlabeled mAb [21, 35] or prior to therapy [32] to obtain pharmacokinetic information. A plot of ^{131}I against time, adjusted for normal physical isotope decay, was then used to determine the kinetics of plasma clearance of the mAb. This approach yielded almost identical elimination half-lives ($t^{\beta}_{1/2}$) of 42 ± 6 h [35], 43.9 ± 8.1 h [21] and 46 ± 2 h [32] respectively. Moreover, the pharmacokinetics of ^{131}I-14G2a indicated one-compartment clearance in both trials. Interestingly, Murray et al., upon analysis of the serum kinetics of unlabeled mAb 14G2a, reported a mean "terminal half-life" of 62 ± 20 h with a range of 7–164 h [32]. These data not only indicate that labeled and unlabeled mAb may have different elimination half-lives with a longer $t^{\beta}_{1/2}$ for unlabeled mAb, but also hint at altered distribution and clearance kinetics of both. Overall, unlabeled mAb 14G2a displays two-compartment whole-body clearance as observed in three out of four phase I trials using this same murine anti-GD2 mAb [16, 32, 37]; however, labeled mAb 14G2a had one-compartment clearance [21, 32, 35].

The serum kinetics of 14G2a in pediatric patients in our study show a considerably shorter β half-life of 18.3 ± 11.8 h compared to the value of 62 ± 20 h for 17 adults and six children in the study done by Murray et al. [32]. Physiological changes, e.g. accelerated metabolism, as well as excretion through the hepatic and renal system [6, 14, 38] may contribute to faster mAb clearance and shorter β half-lives during childhood. Accordingly we recently showed shorter β half-lives of a chimeric anti-GD2 mAb ch14.18 in children compared to adults (Uttenreuther et al. unpublished).

The β half-lives of the first treatment cycle administered to patient 2 and patient 9 were longer than those of their second cycles. Murine mAb are known to be immunogenic and to trigger human anti-(mouse Ig) antibody (HAMA) responses [19, 24, 36]. As will be reported elsewhere, both patients (2 and 9) developed significant immune responses with 309.5 ng/ml and 126.1 ng/ml anti-14G2a reactivity respectively (Huang et al. unpublished). One may speculate that the increased elimination upon retreatment is most likely due to the patients' HAMA and immune complex formation between infused mAb and circulating HAMA [20, 28].

Analysis of our data revealed interesting inter-relationships between several pharmacokinetic parameters. (a) There is a strong correlation between increasing mAb doses infused and mAb peak serum levels achieved. (b) Overall, there is a positive correlation between $t^{\beta}_{1/2}$ and the mAb dose infused (Fig. 3a). (c) Although the peak serum levels increase tremendously with increasing doses (see Table 3), maximum peak levels are reached earlier with doses of at least 250 mg/m^2, as compared to those following mAb doses below 250 mg/m^2. The first two findings were previously determined by us (Uttenreuther et al. unpublished) on mAb ch14.18 and by others [1, 10, 11, 20]. The third finding, a plateau of plasma concentrations after 3 days, may be indicative of reaching steady state at this time. Assuming a β half-life of approximately 18 h, according to the first principle of pharmacokinetics, achievement of steady state would be expected after four half-lives, i.e. after 3 days. Thus our data are consistent with the latter. Moreover, despite an overall correlation between $t^{\beta}_{1/2}$ and mAb dose, following the mAb dose increase from 250 mg/m^2 to 500 mg/m^2 little change in $t^{\beta}_{1/2}$ was observed. Whereas in the absence of Michaelis-Menton drug-elimination kinetics, $t^{\beta}_{1/2}$ should remain constant over the dose ranges, the abrupt increase in β half-life between the

mAb doses of 100 mg/m^2 and 250 mg/m^2 is highly suggestive of saturable elimination for 14G2a. Similar observations were previously documented by Goodman et al. [13], who demonstrated a longer half-life of murine mAb L6 at increasing mAb doses, which leveled off at mAb doses of 200 mg/m^2 or more [13].

Comparison of the β half-lives of murine 14G2a with those reported by us for the chimeric human/mouse mAb ch14.18 (Uttenreuther et al. unpublished) demonstrates the more favorable pharmacokinetics of the human/mouse chimeric mAb. After the first treatment cycle, ch14.18 showed a mean $t^{\beta}_{1/2}$ of 66.6 ± 27.4 h, which is significantly longer than the mean $t^{\beta}_{1/2}$ of 18.8 ± 12.4 h for 14G2a ($P = 0.00003$). Both anti-GD2 mAb had a decreased $t^{\beta}_{1/2}$ upon retreatment.

In short, our study represents the first comprehensive analysis of murine mAb pharmacokinetics in the pediatric population. Several salient features, like (a) a correlation between mAb dose and peak serum levels, (b) dose-dependence of $t^{\beta}_{1/2}$, (c) a decreased $t^{\beta}_{1/2}$ on retreatment, and (d) the more favorable pharmacokinetics of a chimeric antibody revealed by our study, will provide a useful guide in the future design of mAb therapy in children.

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