CHAPTER III

Remarkable pharmacokinetics of monoclonal antibodies: a quest for an explanation

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Monoclonal antibodies (MABs) usually display slow and limited distribution with combined linear and non-linear elimination mechanisms. While studying *individual* pharmacokinetic profiles, it was noticed that MAB plasma concentration can vary abruptly over time, with one or more increases after the time to maximum concentration, when theoretically the concentration should only decline. This chapter summarises the frequency of these additional peaks, and assesses whether normal intra-subject variability and assay variability can explain the observations. For this analysis, a benchmark was used which consisted of three registered (adalimumab, bevacizumab, and trastuzumab) and three unregistered IgG1 MABs.

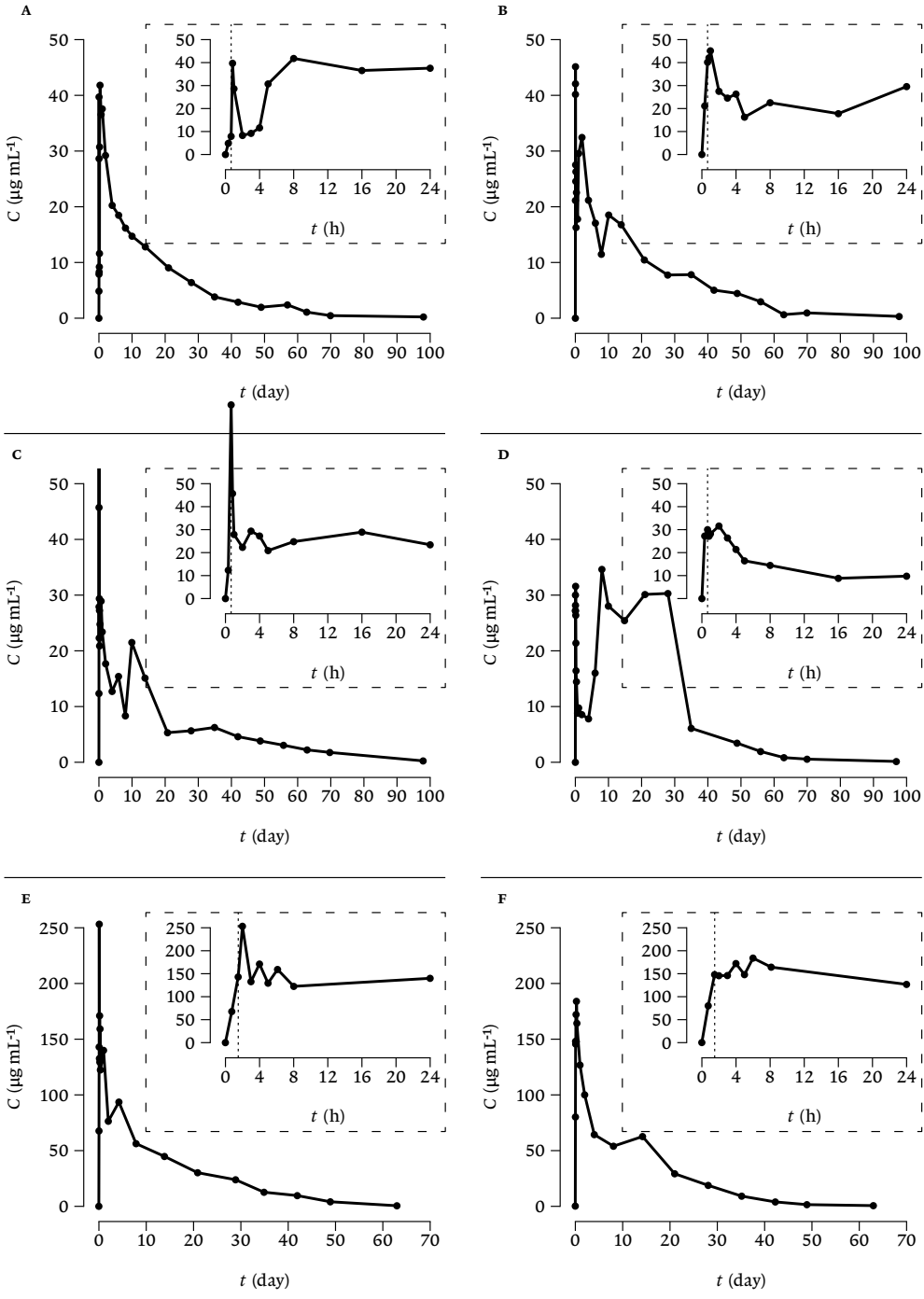
At a selected 'normal' intra-subject variability of 12%, at least 70% of the study participants (approximately 90% for certain MABs) still had at least one additional peak, which decreased when the 'normal' variability was increased. There was no difference in occurrence between the high and low concentration ranges. Only high sample density seemed to be associated with increased likelihood of detecting additional peaks. Based on the analytical variability for the applied ligand-binding assays (5–10%, up to 15% at the lower limit of quantification), the number of observed increases was extremely improbable ($p < 0.01$) for most MABs, especially for the large excursions.

Therefore, the fluctuations are likely genuine. Possible explanations and the relevance for clinical practice are discussed.

Monoclonal antibodies (MABs) are widely used to treat diseases in almost all fields of medicine. They display highly similar pharmacokinetics with a relatively small volume of distribution and a long half-life. Many of the mechanisms responsible for these properties have been extensively studied and are excellently reviewed elsewhere.[1–5]

At Centre for Human Drug Disease (CHDR), multiple clinical trials with MABs are performed annually. When studying their pharmacokinetics, it was noticed that the plasma concentration of MABs in *individuals* can follow a remarkable, or even bizarre, time-course, characterised by (large) excursions (*figure 3.1*), which seems to be in disagreement with current understanding of drug distribution and/or elimination.

Initially, these findings were disregarded as normal intra-subject and assay variability, also because the mean (group) pharmacokinetic profile usually follows a predictable time-course of slow distribution combined with both linear and non-linear elimination. However, after observing fluctuating individual plasma concentrations for an increasing


A
B
C
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C (µg mL-1)
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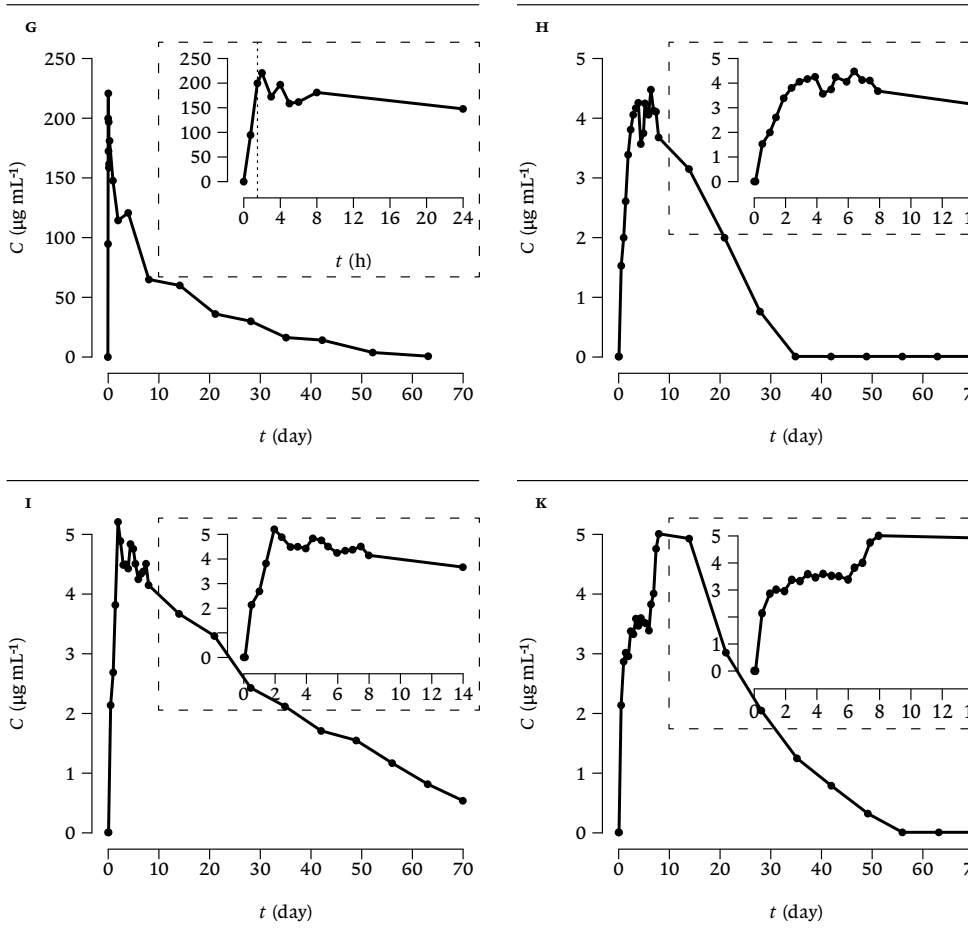

FIGURE 3.1 Individual pharmacokinetic profiles

Representative patterns in individual plasma drug concentrations over time for registered monoclonal antibodies: 2 mg/kg intravenous bevacizumab (A–D), 6 mg/kg intravenous trastuzumab (E–G), 40 mg subcutaneous adalimumab (H–K). The insets depict an enlarged section of the first part of the profile. The dashed lines mark the end of intravenous administration.

TABLE 3.1 Additional peak concentrations after intravenous administration

MAB / PERIOD	VARIABILITY											
	4%		8%		12%		16%		24%		50%	
Dose ≤3 mg/kg												
Bevacizumab ($n=90$)												
Total	88	(97.8%)	87	(96.7%)	83	(92.2%)	72	(80.0%)	65	(72.2%)	41	(45.6%)
<24 h	82	(91.1%)	79	(87.8%)	68	(75.6%)	54	(60.0%)	46	(51.1%)	25	(27.8%)
≥24 h	73	(81.1%)	70	(77.8%)	66	(73.3%)	61	(67.8%)	56	(62.2%)	30	(33.3%)
A ($n=18$)												
Total	18	(100%)	17	(94.4%)	16	(88.9%)	16	(88.9%)	12	(66.7%)	1	(5.6%)
<24 h	18	(100%)	17	(94.4%)	16	(88.9%)	16	(88.9%)	12	(66.7%)	1	(5.6%)
≥24 h	4	(22.2%)	4	(22.2%)	4	(22.2%)	4	(22.2%)	4	(22.2%)	0	
B ($n=33$)												
Total	12	(36.4%)	12	(36.4%)	7	(21.2%)	5	(15.2%)	3	(9.1%)	1	(3.0%)
<24 h	8	(24.2%)	8	(24.2%)	4	(12.1%)	2	(6.1%)	1	(3.0%)	0	
≥24 h	4	(12.1%)	4	(12.1%)	3	(9.1%)	3	(9.1%)	2	(6.1%)	1	(3.0%)
Dose >3 mg/kg												
Trastuzumab ($n=46$)												
Total	46	(100%)	44	(95.7%)	42	(91.3%)	37	(80.4%)	28	(60.9%)	8	(17.4%)
<24 h	45	(97.8%)	42	(91.3%)	39	(84.8%)	33	(71.7%)	22	(47.8%)	3	(6.5%)
≥24 h	21	(45.7%)	21	(45.7%)	20	(43.5%)	20	(43.5%)	16	(34.8%)	5	(10.9%)
C ($n=7$)												
Total	3	(42.9%)	3	(42.9%)	2	(28.6%)	1	(14.3%)	0		0	
<24 h	2	(28.6%)	2	(28.6%)	2	(28.6%)	1	(14.3%)	0		0	
≥24 h	1	(14.3%)	1	(14.3%)	0		0		0		0	

Number (percentage) of subjects with at least one peak concentration other than the maximum concentration (C_{max}) beyond 'normal' intra-subject variability, for which values between 4 and 50% were chosen. Values are displayed per monoclonal antibody (MAB). A separation is made between peaks occurring within the first 24 h from administration and thereafter. A, B, and C are unregistered IgG1 antibodies.

number of MABs, it was considered worthwhile to analyse individual profiles systematically, with the objective to determine whether the fluctuations are genuine. A benchmark was used to assess whether the result could indeed be explained by normal intra-subject and assay variability, or that other factors might be involved.

METHODS

Pharmacokinetic data were taken from clinical trials with MABs in healthy volunteers. Three registered MABs (adalimumab [Humira®], bevacizumab [Avastin®], and trastuzumab [Herceptin®]) were analysed, as well as three unregistered products (denoted A, B, and C). All drugs were IgG1 antibodies and were administered as a single intravenous dose, except for adalimumab, which was administered subcutaneously. The trial participants were healthy subjects, mainly males aged 18–50 years, but some trials included up to 50% females.

All trial procedures were performed in accordance with the different trial protocols. Samples were collected and handled following standard operating procedures. Within each clinical trial, MAB plasma concentration was determined in a single external laboratory, in a single analytical run per participant, using validated methods. Product C was quantitated in batches of samples across multiple subjects, resulting in more than one analytical run per participant.

To determine whether intra-subject variability for MAB plasma concentration would be related to, for example, changes in circulating plasma volume, the time-course of albumin plasma concentration and

erythrocyte counts were studied. As albumin and erythrocytes are produced at a relatively constant rate, and under normal circumstances do not leave the intravascular compartment, these analytes were considered suitable benchmarks.

Samples for albumin concentration and erythrocyte count were always collected concurrently with samples for MAB concentration, albeit at a lesser frequency. On the administration day, albumin and erythrocytes were quantitated 1–5 times, depending on the trial protocol. Thereafter, the ratio of the number of these samples to the number of pharmacokinetic samples ranged between 0.5 and 1. The mean of the individual coefficients of variation (CVs) of albumin concentration per clinical trial ($70 < n < 200$) varied between 3.6 and 4.4% with standard deviations (SDs) of 1.2–1.2 %-point. For erythrocyte count, the mean ranged between 2.7 and 3.7% and the SDs 0.75–1.2 %-point, irrespective if only samples collected on the same day (hours apart) or during the full length of the trial (days to weeks apart) were included in the calculation.

Hence, it appeared that a conservative reference CV (CV_r) of 4% for normal intra-subject variability (CV_i) was justified. It was subsequently investigated whether the observed fluctuations in MAB plasma concentration exceeded 1, 2, or 3 CVs_r, indicating increasing unlikeliness a change in the profile could be explained by 'normal (physiological) variability'. Additionally, we considered a less conservative CV_r of 8%, covering approximately two CVs of the observed variability for albumin and erythrocytes, and a very extreme variability of 50%. By applying this strategy to pharmacokinetic data for different MABs, the number of relative maxima in the concentration-time profile (other than the absolute maximum concentration, C_{max}) that could not be ascribed to the chosen intra-subject variability was counted.

Excursions beyond normal intra-subject variability were identified based on a deviation in the exponential growth constant (λ) outside the margins determined by 1–3 CVs_r (4, 8, and 12%) of the conservative variability estimate or of the less conservative CV_r (8, 16, and 24%). The margins for λ per observation were derived from the formula $A \pm CV_i = B \cdot e^{\lambda t}$, where A is the observed plasma concentration, CV_i the chosen intra-subject variability, B the plasma concentration of the previous sample, and t the difference in sample collection time between A and B. Next, the minimum number of unique λs was determined to describe the observations ($A \pm CV_i$) per individual. A deviation in λ beyond the chosen intra-subject variability was defined as the requirement of two or more unique λs to describe the rising leg of the plasma concentration curve before a relative (local) maximum was reached (see *figure 3S.1* for an example).

This approach assumes linear elimination kinetics, which is known not to be the case for MABs. However, the concentration-time profile of MABs usually approximates linearity at the observed (high) MAB plasma levels in healthy volunteers, where the non-linear elimination mechanism is saturated. Additionally, the non-linearity in elimination manifests as different *negative* λs, whereas the focus of this analysis was on deviations from the surrounding data points in the λ in the rising parts of the concentration profile. Therefore, this approach was considered fit for purpose.

A separation was made between peaks occurring within the first 24 h from intravenous administration – or 14 days from subcutaneous administration – and thereafter. Additionally, the results of the intravenously administered compounds were stratified based on dose.

To study the potential impact of assay variability, the total number of increases between relative

TABLE 3.2 Additional peak concentrations after subcutaneous administration

PERIOD	VARIABILITY					
	4%	8%	12%	16%	24%	50%
Total	128 (98.5%)	118 (90.8%)	90 (69.2%)	72 (55.4%)	54 (41.5%)	19 (14.6%)
<14 days	127 (97.7%)	116 (89.2%)	87 (66.9%)	70 (53.8%)	48 (36.9%)	15 (11.5%)
≥14 days	13 (10.0%)	13 (10.0%)	12 (9.2%)	11 (8.5%)	11 (8.5%)	8 (6.2%)

Number (percentage) of subjects with at least one peak concentration other than the maximum concentration (C_{max}) beyond 'normal' intra-subject variability after a single subcutaneous dose of 40 mg adalimumab ($n = 130$). Values for normal intra-subject variability were chosen between 4 and 50%. A separation is made between peaks occurring within the first 14 days from administration and thereafter.

TABLE 3.3 Probability of increases based on assay variability

MAB / FACTOR (k)		OBSERVED	CV_a 5%	10%	15%	20%	25%
Dose ⩽ 3 mg/kg							
Bevacizumab							
	1.12	228	$<10^{-93}$	$<10^{-5}$	0.86	1.00	1.00
	1.25	153	$<10^{-99}$	$<10^{-37}$	$<10^{-3}$	0.97	1.00
	1.5	78	$<10^{-99}$	$<10^{-99}$	$<10^{-20}$	0.01	0.99
	2	23	$<10^{-99}$	$<10^{-99}$	$<10^{-31}$	$<10^{-7}$	0.20
	3	8	$<10^{-99}$	$<10^{-97}$	$<10^{-37}$	$<10^{-16}$	$<10^{-6}$
	4	3	$<10^{-99}$	$<10^{-59}$	$<10^{-23}$	$<10^{-11}$	$<10^{-5}$
	5	2	$<10^{-99}$	$<10^{-53}$	$<10^{-22}$	$<10^{-11}$	$<10^{-5}$
A	1.12	34	$<10^{-18}$	$<10^{-3}$	0.10	0.39	0.62
	1.25	19	$<10^{-39}$	$<10^{-5}$	0.08	0.64	0.92
	1.5	10	$<10^{-70}$	$<10^{-14}$	$<10^{-3}$	0.16	0.73
	2	1	$<10^{-20}$	$<10^{-4}$	0.05	0.47	0.89
	3	0					
	4	0					
	5	0					
B	1.12	3	0.76	1.00	1.00	1.00	1.00
	1.25	1	0.06	0.99	1.00	1.00	1.00
	1.5	1	$<10^{-6}$	0.14	0.86	1.00	1.00
	2	1	$<10^{-20}$	$<10^{-4}$	0.04	0.38	0.82
	3	1	$<10^{-52}$	$<10^{-12}$	$<10^{-5}$	$<10^{-2}$	0.06
	4	0					
	5	0					
Dose > 3 mg/kg							
Trastuzumab							
	1.12	75	$<10^{-29}$	0.02	0.90	1.00	1.00
	1.25	26	$<10^{-43}$	0.01	1.00	1.00	1.00
	1.5	5	$<10^{-31}$	$<10^{-3}$	0.89	1.00	1.00
	2	1	$<10^{-19}$	$<10^{-3}$	0.14	0.85	1.00
	3	1	$<10^{-51}$	$<10^{-12}$	$<10^{-4}$	0.01	0.21
	4	1	$<10^{-82}$	$<10^{-19}$	$<10^{-8}$	$<10^{-3}$	$<10^{-2}$
	5	0					
C	1.12	3	0.07	0.76	0.94	0.97	0.99
	1.25	0					
	1.5	0					
	2	0					
	3	0					
	4	0					
	5	0					

Probability (p) of finding the observed number of increases ⩾ k in plasma concentration based on the assay's coefficient of variation (CV_a). Only increases after completion of intravenous administration are considered.

extremes ⩾ k in the individual plasma concentration-time profiles was compared to the expected number based on the CV of the used bioanalytical assays (CV_a). Here, k is a factor for which values were chosen as 1.12, 1.25, 1.5, 2, 3, 4, and 5, corresponding to increases between 12 and 400%. A one-tailed binomial test was performed to determine the probability (p) of finding at least the observed number of increases ⩾ k. Samples collected before or during infusion were excluded. This analysis could only be performed for intravenously administered MABs.

The expected number of increases ⩾ k between two consecutive extremes was calculated using the method by Reed *et al.*[6] This approach assumes that the plasma concentration between two samples remains constant, which results in an underestimation of the observed number of increases ⩾ k, as the plasma concentration theoretically declines after the completion of intravenous administration. Because the p-values of increases ⩾ k at any CV_a derived with the binomial tests are thereby overestimated, thus favouring the probability of increases being attributed to assay variability, this methodological shortcoming was accepted.

According to the regulatory guidelines for ligand-binding assays (the type usually applied when measuring MABs in plasma), the CV_a should not exceed 20%, except at the lower level of quantification (LLOQ), where it should not exceed 25%.[7,8] The actual CV_a for the bioanalyses applied in the clinical studies ranged between 5 and 10%, with higher levels (up to 15%) found at LLOQ. Therefore, p-values were obtained at different CVs_a from 5 to 25%.

Data analysis was performed with R (v2.15.2, R Foundation for Statistical Computing, Vienna, Austria, 2012).

RESULTS

Pharmacokinetic observations were available for 130 subjects receiving adalimumab (mean 26.1 observations per subject), 90 subjects receiving bevacizumab (mean 26.3), and 46 subjects receiving trastuzumab (mean 19.8). For products A, B, and C, data were available from respectively 18, 33, and 7 subjects, with a mean number of observations per subject of 18.9, 15.1, and

17.4, respectively. The samples collected during the first seven days after administration summed 16 for adalimumab, 15 for bevacizumab, 12 for trastuzumab, 12 for A, 10 for B, and 10 for C.

Tables 3.1 & 3.2 present the number of subjects with additional maxima beyond increasing CV_i. This shows that with a conservative CV_i of 4% virtually all subjects had an additional peak in their profile. Even if the variability considered normal is increased to 3 CV_r (12%), at least 70% of the study participants (approximately 90% for certain MABs) still had at least one additional peak, with the exception of products B and C. It should be noted, however, that for both products B and C, a sparse sampling scheme was used compared to the other MABs, with less than five samples collected during the first 24 h. This may have limited the chance to identify short-lasting concentration changes.

At a variability of 24%, 60–70% of the subjects who received bevacizumab, trastuzumab, or product A showed an unexplained additional relative maximum, a percentage which decreased further and became more dispersed among the MABs at a variability of 50%. For adalimumab, product B and C, the corresponding numbers were again lower, although the overall pattern observed with increasing intra-subject variability was similar for all investigated MABs. Even when considering an intra-subject variability of 24 or 50% as normal, which is well beyond the variability (CV_r) observed for albumin and erythrocytes, additional peaks remained.

The probability of finding a number of increases with a certain magnitude in plasma concentration rose with increasing assay variability (*table 3.3*). Conversely, the probability was lower for larger excursions. Within the actual CV range for the used ligand-binding assays (5–10%), the number of observed increases was extremely improbable based on assay variability, except for product C. Even at higher CVs_a, which are only accepted at LLOQ (up to 15% for the used assays), assay variability must be considered unlikely in causing the observed increases, especially for those with a large amplitude.

A relationship between standard demographical parameters (age, [lean] body weight, BMI) and the number or magnitude of additional peaks could not be detected, although it should be noted that, as a result of the trial protocols, the populations were highly homogeneous with regard to these parameters. Also, across the different trails, demographical variability was limited.

DISCUSSION

IN THIS CHAPTER, IT IS REPORTED THAT MABs may show unexpected and remarkable pharmacokinetic behaviour, with increases in plasma concentration at the time the compound is cleared. These increases, which are occasionally substantial and long-lasting, appear to be not explained by taking into account physiological or assay variability. There was no difference in occurrence between the high and low concentration ranges. Only high sample density seemed to be associated with an increased likelihood of detecting additional peaks.

When observing fluctuations – especially increases – in the concentration of a drug over time, that theoretically should decline steadily, there are a few explanations to consider. First, pre-analytical errors should be ruled out, such as not disconnecting and removing the infusion material upon stop of intravenous administration, sample switching, applying incorrect dilutions, or calculation errors, *etc.* Subsequently, the assay performance should be considered critically, including, among others, assay precision, within and between-run variability, limit of quantification, and effects of freeze-thaw cycles.

For multiple reasons, assay variability or interference was considered unlikely to explain our observations. First, a vast number of additional peaks was counted (*table 3.3*). Also, the finding that comparable fluctuations were observed for all investigated MABs, in each assay, both in the low and high concentration ranges, and at any moment in time after administration (*figure 3.1*) argues against an assay-related explanation. Furthermore, the data points before or after the peak often confirmed the relatively high concentration, or suggested a steady increase toward the maximum, respectively a decrease following the maximum. These observations are generally not compatible with the randomness one expects to arise from assay variability.

Another explanation to consider is physiological variability, as – for instance – changes in volume status over time may alter the concentration of the MAB in plasma, while the absolute quantity in the body remains unchanged. Fluid shifts were recently postulated by Van Iersel *et al.*[9] as the underlying mechanism for the postural changes in MAB concentration that they had observed. Similar day-to-day variability was seen in our study with adalimumab.

In that clinical trial, pharmacokinetic samples were collected 12 hours apart during the first week (*figure 3.1H–K*). Seemingly, the evening concentrations (0.5, 1.5, 2.5, ... days after administration) were higher than the morning concentrations (1, 2, 3, ... days after administration), with a mean difference of 13.3% (SD 10.5) per participant. It should be noted, however, that for the main part of the adalimumab trial, the participants were ambulatory, and travelled both in the morning and evening to the clinical unit, making postural changes unlikely.

Additionally, the magnitude of many of the remaining fluctuations in plasma concentration for the investigated MABs (*figure 3.1*) exceeded by far the reported increases by Van Iersel *et al.*[9] and what would be physiologically achievable as a result of contraction of the plasma volume. Furthermore, concurrent changes of equal magnitude in intravascularly distributed endogenous substances with a relatively constant production, such as albumin and erythrocytes, was not seen, which is not in keeping with the fluid shift hypothesis. In conclusion, we argue that the majority of the observed fluctuations in the profiles cannot be explained by physiological variability or assay variability and should be considered genuine.

Now that we have demonstrated that the observed fluctuations in MAB pharmacokinetic profiles are likely to be genuine, a few considerations are warranted. First, the occurrence of additional peaks immediately following administration (<24 h and <14 days for intravenous and subcutaneous administration, respectively) was usually higher than in the period thereafter, regardless of the chosen value for normal intra-subject variability. An explanation for this phenomenon may be that the sampling frequency is usually decreased over time, thereby reducing the chance to identify relative extremes. Additionally, some MABs had relatively short profiles, and thus a limited number of data points after 24 h, as was the case for products A and B.

Next, the question rises which physiological mechanism may be responsible for the phenomenon of fluctuating plasma concentration. One explanation comprises the capture and subsequent release of MABs by tissues or components, which would presumably be large quantities of MAB, given the observed magnitude of the excursions, with increases of 50% or more (*table 3.3*). Moreover, the MAB is presumably released quite rapidly, as the changes over time in certain cases approach the infusion rate of intravenous administration (*figure 3.1*). Earlier, we demonstrated the endothelium to be a potential candidate for dynamically binding biopharmaceuticals.[10] Nonetheless, there may be other locations where MABs can be stored temporally. For example, can MABs simply pool in the venous compartment or in less perfused organs? Does an extravascular reservoir exist? Which physiological or pathophysiological mechanisms underlie the release ('auto-injection') of the MAB into the circulation?

Considering daily life, the redistribution of blood flow to various organs during alimentation (gastrointestinal system), resting, and physical exercise (muscles) may either mask or expose sites for adsorption, absorption, and elimination, or, in contrast, flush out pooled or adsorbed MABs in these organs. Possibly, changes in the local milieu (*e.g.* pH), competition for adsorption sites by other substances, and modifications to structural components involved in binding or transport of MABs can mediate the release into the circulation. Without dedicated research on the distribution of MABs over the body, however, these options remain speculative at best.

MABs are designed to specifically bind a particular target, and the resulting complex is internalised and subsequently degraded by either immune cells or the target cell.[1,3] Therefore, this elimination process cannot contribute to increases in the plasma concentration of a MAB. However, it is conceivable that an abrupt decrease in the target-mediated elimination route – for example, because of down-regulation of the target following exposure to an abundance of circulating MABs – can acutely elevate plasma concentration, provided that there is continuous absorption of the MAB into the plasma compartment, as with subcutaneous administration. Other prerequisites for this possible explanation are a relative high absorption rate and a significant contribution of the target-mediated pathway to the total elimination of the MAB, which does not seem to be the case based on published values regarding absorption and elimination rates.[5,11] By analogy, although variations in the absorption rate over time after subcutaneous administration can theoretically change plasma concentrations of MABs, the absorption of MABs from a subcutaneous depot into the circulation is generally understood to be slow,[2] which is not in line with the observed rapid and large excursions.

The neonatal Fc-receptor (FcRn, or Brambell-receptor) requires special consideration. Binding of a mAb to this receptor does not result in lysosomal degradation, but returns the mAb-FcRn complex to the cell membrane.[1,4,5,12] Such recycling of mAbs to the vascular compartment may contribute to fluctuations in plasma concentration, as mAbs can be temporarily sequestered from the circulation.[13] However, research suggests the transportation of immunoglobulins by FcRn is quite rapid.[14] Another function of the FcRn is transcytosis of immunoglobulins, including mAbs. According to current understanding, distribution of mAbs to tissues is slow and limited,[1–3,5] which suggests this process cannot explain our observations. Furthermore, albumin is also a substrate of the FcRn,[12] and comparable fluctuations in its concentration have not been documented. Nonetheless, involvement of the FcRn cannot be ruled out, although it would be interesting to know which factors, in that case, can trigger abrupt changes in FcRn-mediated transcellular transport rate of mAbs.

An important question to be answered is what the clinical relevance of fluctuations in plasma concentration over time could be. Assuming that plasma concentration is a key determinant to achieve therapeutical concentrations at the site of action, measuring and understanding variations in plasma concentrations over time are probably pivotal. Therefore, we hope to initiate a broad discussion within the field on possible explanations for the observed phenomena, as well as how to increase more fundamental knowledge of the pharmacokinetics of mAbs.

In conclusion, the plasma concentration of mAbs can vary abruptly and to a great extent, which cannot be explained by normal physiological or assay variability. Future studies are required to elucidate this phenomenon and to determine its relevance for clinical practice.

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SUPPLEMENT

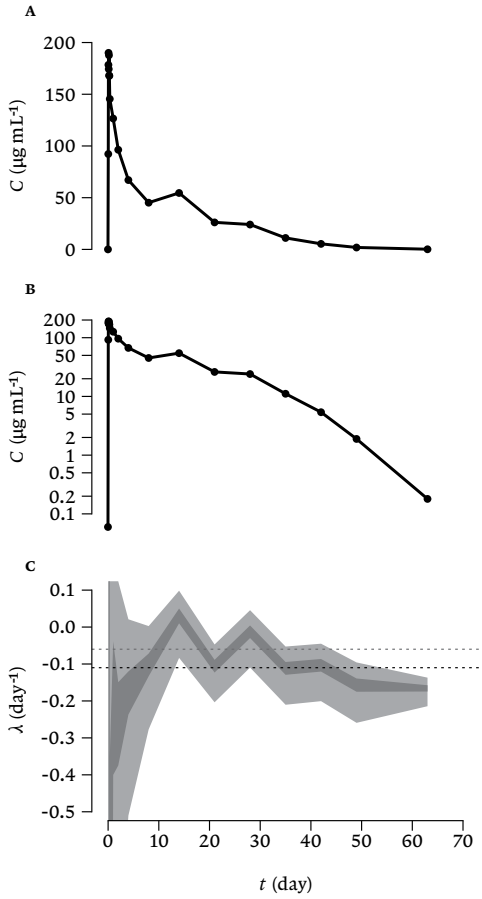

FIGURE 3.51 Example individual pharmacokinetic profile

Example individual pharmacokinetic profile on linear scale (A) and logarithmic scale (B). The margins of the exponential growth constant, λ, are displayed in C and are based on the formula $A \pm \mathrm{CV}_i = B \cdot e^{\lambda t}$, where A is the observed plasma concentration, CV_i the chosen intra-subject variability, B the plasma concentration of the previous sample, and t the difference in sample collection time between A and B. At a CV_i of 8% (dark grey area), no single λ can describe both the relative maximum at day 14 and the surrounding curve (black dashed line). However, at a CV_i of 50% (light grey area), a common λ can be found (grey dashed line). Hence, the additional peak is counted at a CV_i of 8% but not at a CV_i of 50%.