The pharmacokinetics and pharmacodynamics of monoclonal antibodies – mechanistic modeling applied to drug development

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The pharmacology of therapeutic monoclonal antibodies (mAbs) is complex and dependant on both the structure of the antibody and the physiological system that it targets. Patient exposure and responses to mAbs are also related to the structure and activity of mAbs. Furthermore, the pharmacokinetics and pharmacodynamics of mAbs are often inter-related. Pharmacokinetic and pharmacodynamic modeling have been used to elucidate or support the mechanisms of antibodies in development and can be used to identify appropriate dose regimens. Consequently, pharmacokinetic and pharmacodynamic modeling often plays a larger role during the development of therapeutic mAbs than for small molecules.

Keywords mAb, mechanistic modeling, pharmacodynamics, pharmacokinetics

Abbreviations

ADCC antibody-dependent cell cytotoxicity, **APC** antigen-presenting cell, **AUC** area under curve, **CD** cluster of differentiation, **CDR** complementarity determining region, **CTLA** cytotoxic T-lymphocyte-associated antigen, **EGFR** epithelial growth factor receptor, **Fab** antigen-binding fragment, **Fc** constant fragment, **FDA** US Food and Drug Administration, **GvHD** graft-versus-host disease, **HER** human epithelial growth factor receptor, **ICAM-1** intercellular adhesion molecule 1, **Ig** immunoglobin, **IL-2R** interleukin-2 receptor, **LFA-1** lymphocyte function-associated antigen 1, **mAb** monoclonal antibody, **MHC II** major histocompatibility complex class II, **mVar** murine variable, **PASI** Psoriasis Area and Severity Index, **PD** pharmacodynamic, **PK** pharmacokinetic, **RSV** respiratory syncytial virus, **sCD4** soluble CD4 receptor, **sTAC** soluble CD25, **TAC** T-cell activation antigen, **TNF** tumor necrosis factor, **VEGF** vascular endothelial growth factor, **VEGFR** vascular endothelial growth factor receptor

Introduction

Monoclonal antibodies (mAbs) were first shown to have potential for clinically beneficial therapeutic activity in 1982. Philip Karr, a lymphoma patient, demonstrated a complete response to treatment with a tailor-made mouse anti-idiotypic antibody directed against his tumor cells [1]. Since 1992, the number of therapeutic monoclonal antibodies in development and on the market has grown and the class has expanded to cover antibodies, antibody fragments and antibody-fusion proteins. Currently, there are more than 20 antibody products approved for use by the FDA. A summary of the broad characteristics of these agents is provided in Table 1.

In many cases, pharmacokinetic and pharmacodynamic (PK-PD) modeling has expedited the development of these mAbs and has been utilized to support the selection of the dose regimen [2•]. In a recent review, a significant proportion of new drug applications (42 of 244 applications surveyed) included studies of PK-PD modeling of the drug, and of those that did include modeling, more than half were considered critical to the approval and labeling of the drug [3•]. Modeling and simulation evaluation are therefore becoming more commonly used during drug development. A concept used in drug development that has been widely accepted in the pharmaceutical industry and termed the 'learn and confirm' approach benefits strongly from modeling and simulation methodologies [4]. This concept especially benefits from the use of more sophisticated models combining both the concentration-time characteristics (PK) of a candidate drug and the effect-concentration characteristics (PD) of the drug response [5].

The FDA has indicated that PK-PD modeling can be important in the following four areas: (i) to support the use of an approved drug in a new patient population; (ii) to help to understand the relationship between drug concentration and pharmacodynamic response; (iii) to use the drug in a new indication; and (iv) to generate additional information from analysis of drug responses collected during all phases of drug development [6]. PK-PD modeling has been applied to the development of mAbs with increasing frequency.

Background

The development of therapeutic mAbs is largely based on research conducted during the 1960s and 1970s. Several key publications form the basis of the current understanding of the PK and PD of therapeutic mAbs.

The primary structure and the relationship between structure and function of immunoglobulins (Igs) were investigated in the 1960s. The earliest description of their structure and function was in 1966 by Koshland [7]. However, these earlier structural evaluations were somewhat compromised because antibodies purified from human serum from inoculated volunteers were utilized. These samples contained a mixture of antibodies from multiple clones of B-cells (eg, polyclonal antibodies) that interacted with different epitopes. The structural characterization of IgGs was improved by the discovery that patients with multiple myeloma, a monoclonal tumor of antibody-producing plasma cells, often have very high levels of antibodies that react to only one epitope (ie, mAbs) [8]. An additional advance facilitating investigations

Table 1. Listing of currently marketed antibodies and antibody derivatives.

Name	Therapeutic area	Type	Antibody isotype	Target receptor	Pharmacokinetic behavior
Abciximab	Cardiovascular	Fragment	Chimeric Fab: mVar-hIgG1	CD41	Linear
Abatacept	Inflammation	Fusion protein	Extracellular domain of hCTLA-4+hinge of hFc	CD80/CD86	Linear
Adalimumab	Inflammation	mAb	hIgG1	TNFα	Nonlinear
Alefacept	Inflammation	Fusion protein	LFA-3/ hIgG1(Fc)	CD2	Nonlinear
Alemtuzumab	Oncology	mAb	rCDR-hIgG1	CD52	Nonlinear
Basiliximab	Transplantation	mAb	Chimeric: mVar-hIgG1	CD25	Not reported
Bevacizumab	Oncology	mAb	hIgG1	VEGF	Linear
Cetuximab	Oncology	mAb	Chimeric: mVar-hIgG1	EGFR	Nonlinear
Daclizumab	Transplantation	mAb	Hyperchimeric: mCDR-hIgG1	CD25	Linear
Efalizumab	Inflammation	mAb	mCDR-hIgG1	CD11a	Nonlinear
Etanercept	Inflammation	Fusion protein	TNF-receptor/hIgG1(Fc)	TNFα	Linear
Gemtuzumab	Oncology	mAb	mCDR-hIgG4	CD33	Nonlinear
Ibritumomab tiuxetan	Oncology	mAb	Murine IgG1	CD20	Not reported
Infliximab	Inflammation	mAb	Chimeric: mVar-hIgG1	TNFα	Linear
Muromonab-CD3	Transplantation	mAb	Murine IgG2α	CD3	Not reported
Omalizumab	Inflammation	mAb	mCDR-hIgG1	IgE	Linear
Palivizumab	Antiviral	mAb	mCDR-hIgG1	RSV	Not reported
Panitumumab	Oncology	mAb	hIgG2	EGFR	Nonlinear
Ranibizumab	Macular degeneration	mAb	hIgG1κ	VEGF	Not reported
Rituximab	Inflammation	mAb	Chimeric: mVar-hIgG1	CD20	Linear
Tositumomab	Oncology	mAb	Murine IgG2α	CD20	Nonlinear
Trastuzumab	Oncology	mAb	mCDR-hIgG1	Her2	Nonlinear

CD cluster of differentiation, **CDR** complementarity determining region, **CTLA** cytotoxic T-lymphocyte-associated antigen, **EGFR** epidermal growth factor receptor, **Fab** antigen-binding fragment, **Fc** constant fragment, **Ig** immunoglobulin, **LFA-1** lymphocyte function-associated antigen, **mAb** monoclonal antibody, **mVar** murine variable, **RSV** respiratory syncytial virus, **TNF** tumor necrosis factor, **VEGF** vascular endothelial growth factor.

of antibody structure and function was the discovery of a process for generating hybridomas by Kohler and Milstein, which allowed production and isolation of pure mAbs [9].

The general structure for an antibody is illustrated in Figure 1. Antibody monomers are constructed of four polypeptide chains, two heavy chains (C_H) and two light chains (C_L) that are connected by disulfide bonds (-S-S-) at the hinge region. An antibody monomer comprises the following two domains: (i) the variable region, which is specific for the antigen target and is referred to as the Fab or antigen-binding region; and (ii) the constant region, which is referred to as the Fc region. In order to retain favorable PK properties, the Fc region is usually human whereas the Fab region may be murine (chimeric antibodies) or the murine portion may be limited to the hypervariable region (hyperchimeric antibodies). Several different constructs can be made from the monomer, including an engineered monomer, a construct of the Fc and another protein (fusion proteins) or fragments of the binding region (Fab or Fab2). Figure 1 depicts the general structure of an IgG isotype. There are several isotypes of Ig: IgA, IgD, IgE, IgG and IgM. The functions and characteristics of each isotype are listed in Table 2 and summarized by Abbas & Lichtman [10••]. As a consequence of its functionality, the IgG isotype and its derivatives have been the primary focus for therapeutic development.

Brambell postulated that there were receptor-mediated mechanisms that both transmitted immunity from mother to young and protected IgG molecules from catabolism [11••,12,13]. It was recognized that IgG transport- and IgG protection-involved receptors had several common features such as IgG saturation and trans-endosomal transport, acid-enhanced binding and a shared binding site for Fc. Junghans

Figure 1. The general structure of an antibody monomer and its constructs.

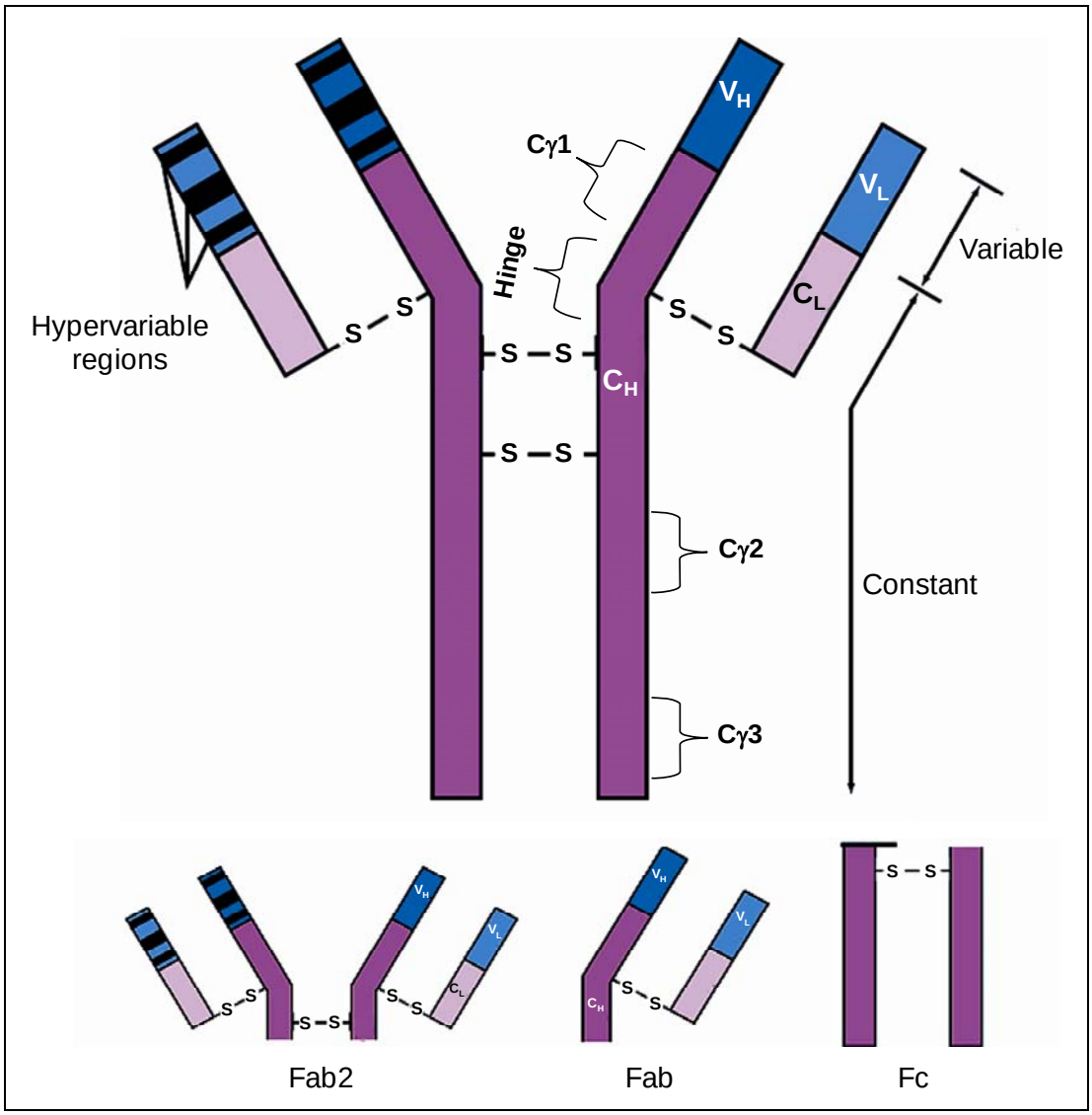


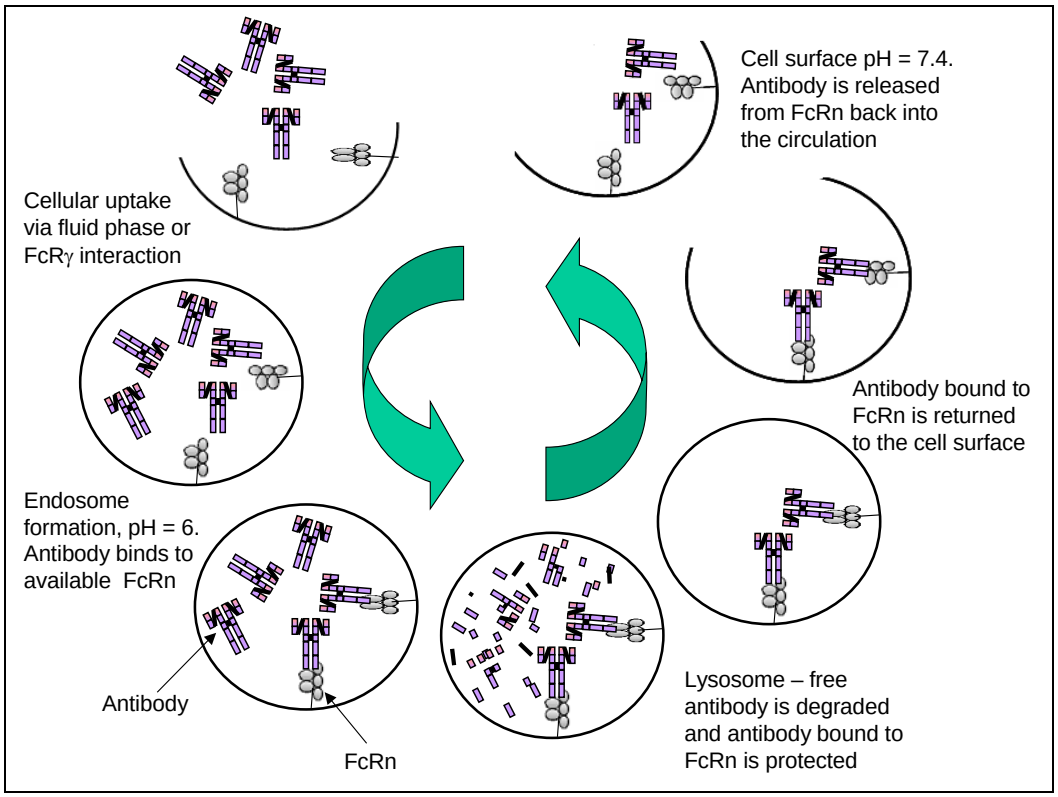
Table 2. Human antibody isotypes and function.

Isotype	Subtypes	Molecular form	Serum half-life (days)	Function
IgA	1 and 2	Monomer, dimer	6	Mucous immunity
IgD	None	Monomer	3	B-cell antigen receptor (function unknown)
IgE	None	Monomer	2	Mammalian hypersensitivity
IgG	1 to 4	Monomer	23	Opsinization, complement activation, antibody-dependent cell cytotoxicity, neonatal immunity, feedback inhibition of B-cells
IgM	None	Pentamer, hexamer	5	B-cell antigen receptor, complement activation

later proved that these receptors were the same [14,15]. This receptor is often referred to as FcRn in reference to neonatal rat intestine (the tissue from which it was initially cloned), and is also called FcRB or the 'Brambell receptor' after the scientist who first postulated its existence. A simplified schematic of FcRn-mediated antibody salvage is provided in Figure 2.

FcRn is expressed in relevant tissues involved in immunity transmission. In humans, IgG transmission is antenatal, and FcRn is expressed in human placental syncytiotrophoblasts and therefore is not highly expressed in newborn intestinal epithelium. In adults, FcRn is largely expressed in the vascular endothelial cells, the most endocytically active tissue in adults. FcRn is also detectable at lower levels on

Figure 2. Schematic of FcRn antibody salvage.



monocyte cell surfaces and on a small subset of tissue macrophages that express high levels of FcRn on their cell surface [16].

In 1965, Spiegelberg *et al* determined that the half-life of the Fc fragment was similar to that of an intact antibody, whereas the Fab fragment was cleared quickly [17]. Building on this and other observations, Morrell *et al* evaluated the pharmacokinetic behavior of the different subclasses of IgG antibodies, reporting that the average biological half-life of IgG1, IgG2, and IgG4 was 21 days, while that of IgG3 was only 7.1 days [18]. It was also observed that although IgG3 has a shorter half-life, the effect of high concentrations of IgG3 results in IgG3 inducing similar overall effects as those of other subtypes. In this important paper, the authors noted that the PK behavior of different subclasses of IgGs was dependent on the structure of the Fc region. The authors also evaluated the distributional behavior of the subclasses, and determined that the vascular compartment contained 51 to 54% of the total body pools of IgG1, IgG2, and IgG4, and 64% of the total body pool of IgG3, suggesting that antibodies were not confined to the vascular compartment. Finally, based on data from multiple myeloma patients, the authors showed that elevated serum concentrations of any IgG subclass were associated with a substantially shortened biological half-life, yielding an inverse relationship between serum IgG concentration and elimination half-life (Figure 3).

Clearly, the structural integrity of the Fc portion plays a central role in mAb clearance, primarily by allowing the antibody to be recycled or salvaged by FcRn. The clearance of antibodies is primarily via proteolytic catabolism; however, there is also a basis for the concept of receptor-mediated clearance of these molecules [19••,20]. This mechanism has been proposed for several antibodies.

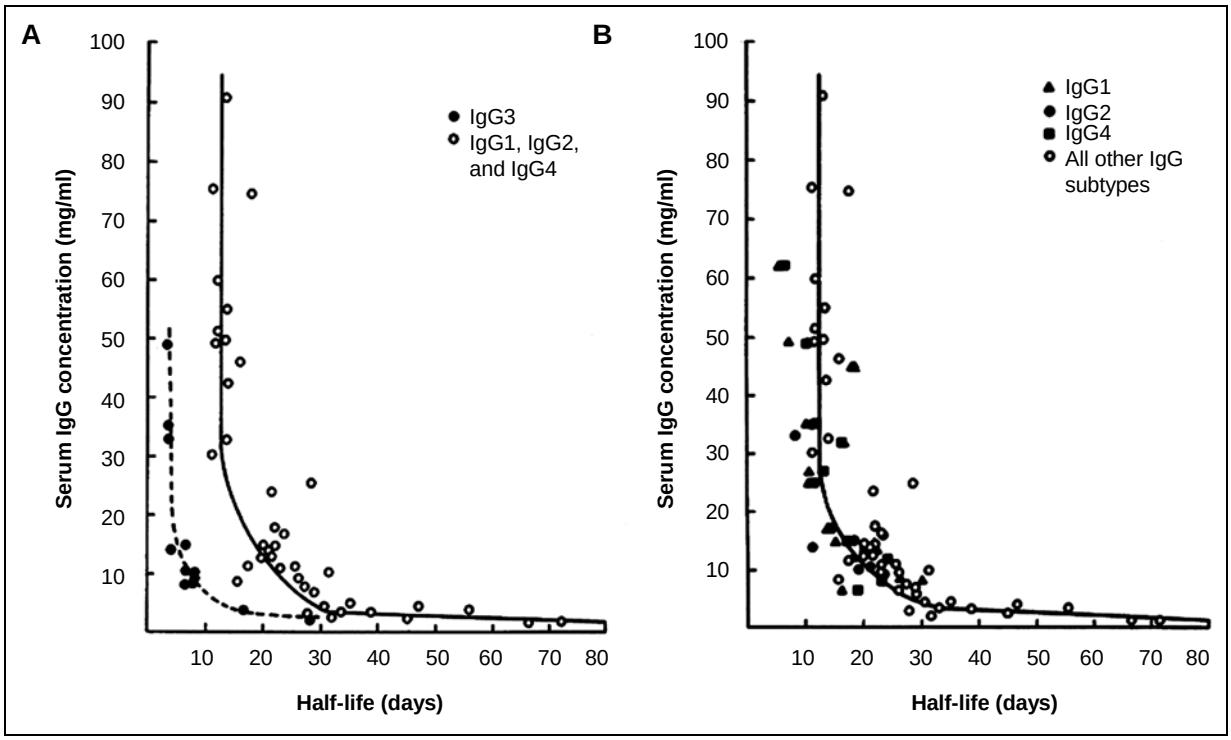
There have been a number of other good published reviews of the pharmacology, PK and PD of mAbs [21,22,23•,24,25], and all point to the fact that investigations of the PK and PD of therapeutic mAbs are complex in nature. Some of the complexities of these evaluations are presented in the examples given below.

Daclizumab

Daclizumab is an engineered IgG1 therapeutic mAb comprising 90% human and 10% murine antibody sequences. Daclizumab binds specifically to CD25 (or T-cell activation antigen [TAC], the 55-kD α-chain of the interleukin-2 receptor [IL-2R]), which is expressed on the surface of activated lymphocytes. CD25 is upregulated on activated T-lymphocytes in several autoimmune diseases and in patients with allograft rejection or graft-versus-host responses [26]. CD25 is also overexpressed in several hematological malignancies.

The PK of daclizumab in patients who had previously undergone bone marrow transplant and subsequently developed graft-versus-host disease (GvHD) were first published in 1994, and the mean half-life values estimated from these data are listed according to dose in Table 3 [27]. GvHD is a form of an autoimmune disease where the transplanted donor bone marrow reacts against the host.

Figure 3. Graphs to show the relationship between antibody concentration and half-life.



(**A**) A comparison of pharmacokinetic behavior of IgG3 versus all other IgG subtypes. (**B**) Plot of concentration versus half-life for IgG subclasses 1,2 and 4.
(*Figure derived from reference [18].*)

Table 3. Mean half-life of daclizumab by dose in patients with GvHD.

Dose (mg/kg)	Number of patients	Half-life (h)
0.5	4	79
1	4	88
1.5	12	94

(*Adapted with permission from the American Society of Hematology and Anasetti C, Hansen JA, Waldmann TA, Appelbaum FR, Davis J, Deeg HJ, Doney K, Martin PJ, Nash R, Storb R, Sullivan KM et al:* ***Treatment of acute graft-versus-host disease with humanized anti-Tac: An antibody that binds to the interleukin-2 receptor.*** *Blood (1994)* ***84****(4):1320-1327. © 1994 American Society of Hematology.)*

The lower limit of assay quantitation used for daclizumab was 7.8 μg/l, although the concentrations were generally measurable for 5, 30 and 42 days following dosing at the three concentrations listed in Table 3, respectively. There was a slight trend of increasing half-life with increasing dose, although the number of treated patients was small and the parameter estimates were variable. No attempt was made by the authors to correlate the estimated terminal half-life with the receptor-positive cell counts, but the concentration-time profile from the first and last doses were similar, indicating no evidence of time-dependent changes in PK.

The PK profile of daclizumab in patients undergoing renal transplant was different to that seen in patients with GvHD [28]. In renal transplant patients, the terminal half-life of daclizumab was 273 h, substantially longer than any previously reported half-life for the drug. Population PK analysis of the data obtained from all renal transplant patients in this study (n = 123) was performed using a simple linear two-compartment model, generating the following values for a reference patient (45-year-old male Caucasian patient with a body mass of 80 kg and no proteinuria): daclizumab clearance was 0.015 l/h, volume of distribution of the central compartment was 2.5 l, and volume of peripheral compartment was 3.4 l [29]. The estimated terminal elimination half-life for the reference patient was 480 h (20 days), which is longer than was reported in the earlier publication, in this population. Bayesian estimates of terminal elimination half-life ranged from 264 to 912 h. Evaluations for influential covariates determined only body weight to be important, although, again, receptor-positive cell counts were not assessed. However, within this analysis the low inter-patient variability for clearance and central volume of distribution were low (15 and 27%, respectively), making it unlikely that additional covariates would contribute substantially to the variability in exposure conditions.

In evaluating the PK data of daclizumab across different indications, it is evident that clearance is dissimilar in different patient populations, although the distribution volume is fairly consistent for all patients. A listing of published parameters for daclizumab in three different patient populations is provided in Table 4.

Table 4. Summary of pharmacokinetic parameters for daclizumab in different patient populations.

Parameter (unit)	Indication		
	Treatment of GvHD	Prevention of GvHD	Renal transplant
Clearance (l/h)	0.042	0.0314	0.015
Vd_{ss} (l)	5.81	6.91	5.9[a]
Half-life (h)	79 to 94	165.4	480

[a]value taken to be the sum of the typical volumes of distribution for the central and peripheral compartments. **GvHD** graft-versus-host disease, **Vd_{ss}** volume of distribution at steady-state.
(*Table derived from reference [30].*)

Patients undergoing treatment for GvHD would be expected to have a substantially higher concentration of CD25+ T-cells than either patients undergoing renal transplant or patients administered daclizumab prophylactically for prevention of GvHD: the trends seen in Table 4 support this hypothesis. Consequently, in patients with hematological malignancies, who are known to overexpress CD25, daclizumab would be expected to have a similar or faster clearance and a similar or shorter half-life than in GvHD patients. A study recently published by Koon *et al* determined a strong inverse correlation between CD25+ cells expression and apparent daclizumab half-life [31]. By comparing the percentage of daclizumab remaining in serum at 48 h post-dose with a 'CD25 index', which was a summary score calculated to represent the entire CD25 tumor burden, this research team found that daclizumab clearance was statistically significantly correlated to CD25 expression. A listing of individual parameters and associated disease characteristics for daclizumab in patients with leukemias expressing CD25 is presented in Table 5.

Koon *et al* also showed a strong correlation between tumor volume and the PK of daclizumab [31]. Several patients in this study received ^{111}In-labeled daclizumab and underwent whole body imaging. In patient 10, who had a low tumor volume, daclizumab remained largely in the vascular space. Conversely for patient 1, who had a high tumor volume, the antibody cleared from the blood compartment rapidly and localized to tumors in the bone marrow and spleen. Patient 7 also displayed rapid clearance following the first dosing of daclizumab, with more than 80% of the dose having been cleared within 48 h. After repeated dosing, the PK of the drug appeared to follow the more typical two-compartment behavior and the estimated β half-life stabilized at approximately 480 h.

In addition to the primary-targeted cell surface CD25 receptor, the presence of solubilized or shed receptors (ie, antigenemia) must be considered. The shedding of receptors is common in several cancers (eg, the shedding of human epidermal growth factor receptor 2 [HER2/neu] in breast cancer), therefore attention should be given to the binding interactions between the administered antibody and these receptors. Junghans *et al* reported that soluble CD25 (sTAC) can block daclizumab binding sites and diminish antibody binding [32]. This study concluded that the *in vivo* activity of

Table 5. Pharmacokinetic parameters and disease characteristics for daclizumab in patients with CD25-expressing leukemias.

Patient	α half-life (h)	β half-life (h)	Percentage of initial dose at 48 h	WBC count	CD25+ tumor cells (%)	Bone marrow involvement	Index of CD25+ cell count at baseline	sTAC (U/ml)
1	12.48	Not available*	13	40,000	92	5	5.70×10^{17}	68,800
2	17.52	309.6	48	26,900	100	4	7.20×10^{16}	5720
3	23.52	160.8	42	2600	31	5	2.10×10^{15}	3620
4	47.52	Not available*	29	144,800	53	Not available	1.30×10^{16}	8250
5	34.56	Not available*	44	92,600	91	3	6.40×10^{15}	13,000
6	8.64	180	49	18,800	50	5	1.90×10^{15}	945
7	28.32	Not available*	18	341,000	100	4	5.50×10^{16}	1210
8	18	456	34	8600	40	2	9.50×10^{15}	1900
9	72.24	1012.8	75	30,700	82	2	7.20×10^{14}	1240
10	34.56	648	66	6100	82	1	7.60×10^{14}	497

sTAC soluble CD25, **WBC** white blood cell. *Serum concentrations of daclizumab decreased rapidly, preventing the determination of a β phase half-life.

daclizumab was inversely correlated with the sTAC concentrations, although tumor targeting could be achieved in the presence of excess sTAC. Because daclizumab has a fully human Fc, it undergoes salvage via FcRn and therefore sTAC has no impact on the PK of daclizumab, although the presence of sTAC reduced free concentrations of daclizumab.

Clenoliximab

Clenoliximab is a chimeric macaque/human mAb of the IgG4 isotype that targets CD4+ T-cells. *In vivo*, clenoliximab inhibits antigen-induced T-cell proliferation, lymphokine-release and helper T-cell functions by inhibiting alternative, non-receptor-mediated pathways for triggering T-cells [33]. The CD4 molecule has a complex regulatory function in T-cell activation and mAbs against CD4 were expected to exert their activity through several possible mechanisms, such as the reduction of the CD4+ T-cell population or interference with the CD4 interaction with the major histocompatibility complex class II (MHC II), or a combination of both.

Clenoliximab has the same variable region as keliximab, an IgG1 mAb that was evaluated for treatment of rheumatoid arthritis in clinical trials but which caused variable CD4 T-cell depletion in an asthma study [34] and in a rheumatoid arthritis study [35]. Totoritis *et al* noted that for both studies, there was a dose-dependent increase in CD4+ T-cell coating with keliximab, but that the pattern of CD4 depletion was variable [35]. Furthermore, CD4+ T-cell coating with keliximab did not appear to correlate with CD4 depletion.

Therefore, in order to create a mAb that lacked the immunosuppressive characteristics of keliximab, clenoliximab was generated by attaching the variable domains of a cynomolgus macaque antibody to human Fc regions. The heavy chain constant region comprised a modified IgG4 chain containing two single residue substitutions designed to ablate residual Fc receptor-binding activity and to stabilize heavy chain dimer-formation. Clenoliximab showed reduced binding to Fc receptors, suggesting that it could not mediate antibody-dependent cell cytotoxicity (ADCC) or modulation/loss of CD4 from the surface of T cells, unless a rheumatoid factor or activated monocytes were present. This resulted in a reduced potential to deplete CD4+ T-cells *in vivo* while inhibiting T-cell activation through antigen coating and down-modulation [36]. Clenoliximab was evaluated in several *in vivo* and *in vitro* studies, where receptor modulation was observed, but T-cell depletion was not evident [37].

Sharma *et al* reported the results of a PK-PD modeling study for keliximab and clenoliximab in transgenic mice [38]. The PK model used was a two-compartment model with nonlinear clearance from the central compartment, and first-order transfer and nonlinear elimination from the peripheral compartment. An indirect effect PD model was used to describe the relationship between clenoliximab and keliximab concentrations and changes in circulating CD4+ cells and to the cell-surface receptor density on circulating T-cells. An E_{max} (maximum effect) function for drug effect within the indirect effect response model (incorporating S_{max} [the maximum stimulatory effect of the antibody] and SC_{50} [concentration of the antibody required at half maximal response] parameters) was used to assume each antibody's PD effects through a stimulatory mechanism for response markers to both CD4 T-cell depletion and CD4 down-modulation. A comparison of these E_{max} function parameters for each antibody for the two markers is presented in Table 6.

The models used by Sharma *et al* described the PK data well, and facilitated a quantitative comparison of the potency of keliximab and clenoliximab to deplete CD4+ T-cells and downregulate CD4 receptor expression. Evaluation of the down-modulation of the CD4 receptors by the two antibodies required the maximum effect parameters to be fixed but did allow a comparison of the potency of each antibody to reduce the CD4 receptor density. As expected, both antibodies showed comparable potency in modulating CD4 receptor density, but keliximab exhibited greater potency to induce clearance of CD4+ T-cells, presumably by ADCC [38].

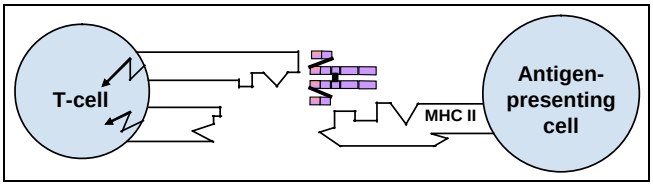
The PK and PD of clenoliximab were evaluated in patients with moderate or severe rheumatoid arthritis using a population-based approach [39]. Mould *et al* reported that clenoliximab displayed nonlinear PK behavior and caused an 80% reduction in CD4 density for up to 3 weeks without depleting T-cells. The authors used a two-compartment model with nonlinear elimination from the central compartment to describe the PK of clenoliximab, and then developed a model describing the relationship between bound and free antibody via antigen-coating effects. The bound antibody concentration was then used to inhibit the CD4 receptor density using an indirect effect PD model. The PK, binding and PD models were then used to anticipate the effects of clenoliximab in untested regimens and to optimize the design of future clinical trials of clenoliximab. The authors indicated that it was not possible to determine if the presence of clenoliximab inhibited the synthesis of new receptors or stimulated receptor-loss. Although CD4

Table 6. Comparison of estimated pharmacodynamic parameters for keliximab and clenoliximab for CD4 depletion and down-modulation.

Parameter	Keliximab	Clenoliximab
S_{max} for CD4 T-cell depletion	28.2	16.2
SC_{50} for CD4 T-cell depletion (ng/ml)	37,500	419,000
S_{max} for CD4 down-modulation	0.54 (fixed)	0.40 (fixed)
SC_{50} for CD4 down-modulation (ng/ml)	54,100	59,400

internalization has been reported in response to stimuli that activate T-cells, this mechanism seemed unlikely in the present setting given that there were no such stimuli [40]. Figure 4 presents a schematic diagram of the potential mechanisms for clenoliximab activity. In the absence of antibody, the antigen-presenting cell (APC) binds to the receptor on the T-cell. However, the presence of antibody either prevents binding or causes receptor binding to be non-productive. These interactions can result in either an inhibition of new receptor formation, stimulation of the loss of existing receptors, or a blockage followed by internalization or downregulation of the receptors.

Figure 4. Potential mechanism for clenoliximab activity at CD4 receptors on T-cells.



MHC II major histocompatibility complex class II.

In order to elucidate the mechanistic activity of clenoliximab, Hepburn *et al* investigated the formation of soluble CD4 receptor (sCD4) in patients treated with clenoliximab [41]. The authors reported that maximal CD4 coating (100%) and maximal down-modulation (up to 30% of baseline) of the receptor were maintained by treatment with clenoliximab. Furthermore, sCD4-clenoliximab complex accumulated over time until clenoliximab was cleared from the circulation, after which sCD4 was rapidly cleared. The formation of soluble antibody-receptor complexes has been reported previously for daclizumab, when the antigen exists in both cell-associated and soluble forms. However, the CD4 receptor is not normally soluble, making this study to be the first to demonstrate soluble antibody-antigen complex formation following exposure to an antibody.

Based on an assumed 1:1 antibody-receptor stoichiometry and the measured concentrations of sCD4, the authors determined that much of the administered dose of clenoliximab was involved in receptor shedding. Therefore, the down-modulation of CD4 was thought to be caused by antibody-mediated stripping of CD4 from the T-cell surface.

Efalizumab

Efalizumab is a recombinant humanized monoclonal IgG1 antibody marketed for the treatment of moderate to severe chronic plaque psoriasis [42]. Efalizumab binds to the CD11a subunit of lymphocyte function-associated antigen 1 (LFA-1), preventing LFA-1 binding to intercellular adhesion molecule 1 (ICAM-1) [42]. LFA-1 is a leukocyte cell surface glycoprotein that promotes intercellular adhesion in immunological and inflammatory reactions. It is an αβ integrin complex that is structurally related to receptors for extracellular matrix components, and thus belongs to the integrin family [43]. The interaction between LFA-1 and ICAM-1 is important to stabilize the immunological synapse that forms between T-cells and APCs; this interaction also mediates T-cell binding to endothelial cells [44]. Binding to human T-cells induces a rapid down-modulation of cell surface CD11a receptor and inhibits T-cell activation, migration, and trafficking *in vivo* [45]. By blocking this interaction, the T-cell-mediated inflammatory response is abrogated. Several studies have documented the clinical efficacy of efalizumab in moderate to severe psoriasis [46,47].

The clearance for efalizumab was reported by Bauer *et al* to be 322 ml/day following a single dose of 0.3 mg/kg; however, at higher doses of 3 and 10 mg/kg the clearance was reported to be substantially faster (11 and 6.6 ml/day, respectively) [48••]. The authors related CD11a expression to efalizumab clearance and postulated a receptor-mediated mechanism for efalizumab. A year later, Gottlieb *et al* reported that efalizumab dosed at > 0.3 mg/kg saturated CD11a binding sites in human psoriasis patients [49]. Consequently, efalizumab doses of ≤ 0.3 mg/kg did not completely saturate or down-modulate CD11a cell surface expression.

As mentioned previously, the concept of receptor-mediated clearance of therapeutic mAbs has been proposed for several agents. In 1994, Gaeitta *et al* reported a method to measure the internalization of two epithelial integrin heterodimers, α6β1 and α6β4, induced by cross-linking with specific antibodies [50]. Later research by Leone *et al* with an anti-rat antibody (intergrin α4 antibody, TA-2) also showed internalization of the receptor-antibody complex [51]. The authors reported that TA-2 caused a decrease in α4 integrin expression on the cell surface, which resulted from internalization of integrin α4/TA-2 complexes.

Several evaluations were undertaken to verify that efalizumab undergoes receptor-mediated clearance. This theory was evaluated by Coffey *et al* using both efalizumab and human blood T-cells, as well as using the mouse surrogate antibody muM17 and T-cells purified from mouse lymph nodes [52]. In the *in vitro* systems tested, the authors demonstrated that after initial antibody-receptor binding, internalization occurred but was dependent on cross-linking with a secondary antibody. The authors reported that the clearance of efalizumab was inhibited by concanamycin A, an H+-ATPase inhibitor that attenuates lysosomal function, suggesting an important role for lysosomes in the intracellular clearance of anti-CD11a antibody. *In vivo* research by Coffey *et al* demonstrated that muM17 is primarily distributed to leukocytes and macrophages in the peripheral blood, spleen and liver, with uptake in the lymph nodes and bone marrow [53]. At least a portion of the antibody was internalized and cleared by peripheral monocytes, lymphocytes and splenocytes, and was transported to lysosomes for degradation. Other research by Buddenkotte *et al* demonstrated that CD11a does not undergo receptor shedding [54].

During the clinical development of efalizumab, PK-PD models were developed to characterize its PK and PD profile of CD11a expression on CD3+ lymphocytes. Initially, two different PK-PD models were developed using chimpanzee

PK and PD data obtained after intravenous administration of the drug [48••]. In this evaluation, both models provided good fits with the data. The PK chimpanzee model incorporated a Michaelis-Menten clearance term to account for the fact that the concentration-time profile of the drug suggested that antibody clearance had reached saturation at concentrations above 10 μg/ml. In the PD model CD11a cell counts were accounted for in the Michaelis-Menten clearance term to be more consistent with the proposed receptor-mediated clearance hypothesis. Both models utilized an indirect-response system to describe the change in CD11a+ cell counts after exposure to efalizumab. The PD model indicated the rapid reduction in CD11a expression on CD3+ lymphocytes, which declined to approximately 25% of pretreatment levels regardless of the administered dose of efalizumab. Cell surface CD11a levels remained reduced for as long as efalizumab was detectable, after which the expression of CD11a returned to normal within 7 to 10 days post treatment. The PD model also indicated that CD11a saturation was achieved at concentrations of approximately 10 μg/ml. A population PK-PD approach was used to characterize the data from dose-ranging studies in humans. Based on the results obtained by fitting both models to the available data, it was not possible to determine which model was more appropriate; however, the PD model was selected as being more mechanistically functional as it described the interaction of efalizumab binding to CD11a, resulting in the removal of efalizumab from the circulation and reduction of cell-surface concentrations of CD11a. The PD model accounted for the continually changing number of CD11a molecules available for clearing efalizumab from circulation based on each individual's prior exposure of cells expressing CD11a to hu1124 [48••]. A similar PK-PD model was also developed and implemented for muM17, the murine analog of efalizumab, in order to improve study designs in murine models [55].

A later evaluation of clinical data expanded the mechanistic PK-PD model to relate the changes in CD11a to clinical response (efficacy) [56]. For this analysis, the psoriasis area and severity index (PASI) was used to measure efficacy, and the percentage change of predose CD11a was used as the PD measurement. In the efficacy model, the rate of psoriasis skin production was assumed to be directly proportional to the amount of free-surface CD11a on T-cells. The growth of psoriasis was offset by the rate of skin healing. An additional CD11a-independent component to psoriasis skin production accounted for incomplete response to efalizumab therapy, and the final model described all PK and PD data reasonably well.

A population PK study of efalizumab using data from pivotal trials in psoriasis was much less complex than the models used for earlier development of this compound [57]. This evaluation characterized the PK of efalizumab in 1088 individuals with moderate to severe psoriasis who received 1- or 2-mg/kg/week subcutaneous efalizumab doses for 12 weeks. The majority of the concentration data were obtained from three phase III clinical trials with only day 42 and/or day 84 trough levels, which necessitated the use of a one-compartment model with first-order absorption and elimination. The population means for the volume of distribution, absorption rate constant and apparent clearance were 9.13 l, 0.191/day and 1.29 l/day respectively, for a typical individual receiving a 1-mg/kg dose. Inter-individual variability in clearance (CL/F) was 48.2%. Body weight was the only influential covariate identified in this evaluation. Other covariates, that is, obesity, baseline lymphocyte counts, PASI score and age had only modest effects.

Antibodies directed against EGF and VEGF

EGF is a growth factor that regulates cell growth, proliferation and differentiation [58]. The interaction between EGF and its receptor, EGFR, stimulates the intrinsic protein-tyrosine kinase activity and internalization of EGFR, resulting in mitogenic signal transduction [59]. Experimental evidence suggests that aberration of EGFR-mediated signal transduction plays a critical role in tumorogenesis and tumor growth [60].

The development of new blood vessels (angiogenesis) requires stimulation of vascular endothelial cells through the release of angiogenic peptides. Vascular endothelial growth factor (VEGF) is the most potent of these peptides, causing a tyrosine kinase-signaling cascade on binding to VEGF receptor (VEGFR), which initiates the production of factors that stimulate vessel permeability, proliferation, survival, migration and, finally, differentiation into mature blood vessels. As implied by its name, VEGF activity is restricted primarily to vascular endothelial cells.

VEGF and EGF have some common characteristics. VEGF is frequently expressed by invasive breast cancers [61] and several studies have documented VEGF expression to correlate with poor prognosis in both node-positive and node-negative breast cancer patients [62]. Similarly, the expression, overexpression, or aberrant function of EGFR is characteristic in several solid tumors including renal, lung, breast and colorectal tumors [63].

ABX-EGF

Rowinsky *et al* investigated the safety PK and activity of ABX-EGF, an EGFR-targeted antibody [64]. Non-compartmental evaluation of the serum ABX-EGF concentrations showed greater than dose-proportional PK behavior, which the authors attributed to progressive saturation of the EGFR sink. Since the receptor-antibody complex was expected to be internalized in this environment, the assumption that the nonlinear behavior is due to receptor saturation is reasonable. These data were modeled using a two-compartment model with both linear and nonlinear clearance from the central compartment; this model described the data well. From the model parameter estimates, the half-life of ABX-EGF (which was derived as a secondary parameter expected when the nonlinear clearance pathway is fully saturated) was, on average, approximately 16 days. The PK modeling was used to help determine the concentrations required to saturate EGFR in this clinical setting.

Table 7. Single-dose pharmacokinetic parameters for cetuximab.

Cetuximab dose (mg/m^2)	C_{max} (mg/ml)	AUC (mg.h /ml)	Half-life (h)	Clearance (l/h)
50	21.8	748	24.9	0.127
100	49.7	2909	41.2	0.08
250	149	13,072	74.6	0.038
400	221	23,217	90.6	0.039
500	230	23,029	94.7	0.05

(*Adapted with permission from Prous Science and Harding J, Burtness B:* ***Cetuximab: An epidermal growth factor receptor chimeric human-murine monoclonal antibody.*** *Drugs of Today (2005)* ***41****(2):107-127. © 2005 Prous Science.*)

2F8

The antibody 2F8 (a human IgG1κ isotype being developed by Genmab A/S), which is targeted against EGFR, has similar PK behavior in cynomolgus monkeys as ABX-EGF [65]. The researchers of this study noted that receptor-mediated antibody internalization affected the PK behavior and dose-effect relationship of the antibody. The clearance was assumed to be receptor-dependent and was included in a PK model to account for the observed nonlinear behavior. The researchers evaluated two models: a two-compartment model with parallel linear and nonlinear clearance from the central compartment; and a two-compartment model with linear clearance from the central compartment and binding-mediated clearance from the peripheral compartment to describe the concentration-time data. The binding-mediated clearance model was preferred because it produced more physiologically reasonable results and described the data adequately, although there was some overestimation of the lower concentration values.

Cetuximab

Cetuximab is a chimeric human-murine mAb that binds to EGFR. It was approved by the FDA in February 2004 to be used in combination with irinotecan for the treatment of EGFR-expressing, metastatic colorectal cancer in patients who had failed to improve with initial irinotecan-based chemotherapy. Cetuximab was also approved for administration as a single agent in the treatment of patients with EGFR-expressing, metastatic colorectal cancer who are intolerant to irinotecan-based chemotherapy. However, clinical activity of cetuximab has been reported in tumors that do not express EGFR [66].

Cetuximab activity is thought to occur via binding to EGFR, which prevents stimulation of the receptor by endogenous ligands, resulting in the loss of EGF activity. Binding of cetuximab to the EGFR also results in internalization of the antibody-receptor complex, leading to the downregulation of EGFR expression, a mechanism that is consistent with other EGFR-targeted antibodies [67]. Consequently, the mechanism of clearance of cetuximab is reported to be via internalization of the cetuximab-EGFR complex primarily on tissues with high EGFR expression, for example, hepatocytes and skin.

The PK behavior of cetuximab has not been extensively published, although there have been several presentations of this topic at various meetings. Cetuximab has been shown to exhibit nonlinear PK when used as monotherapy or in combination with radiotherapy [68] or with chemotherapy [69]. A listing of the non-compartmental PK parameters determined for single doses of cetuximab is presented in Table 7 [67]. Phase I dose-finding clinical trials demonstrated that continuous saturation of cetuximab clearance (AUC) was achieved in the majority of patients using an initial loading dose of 400 mg/m^2 followed by 250 mg/m^2 weekly [70]. The terminology used to describe the PK behavior of cetuximab is not clear, but assuming that the PK behavior of cetuximab is consistent with other related antibodies (ie, nonlinear), then the recommended dose regimen should be able to maintain concentrations sufficient to saturate EGFR in the target patient population.

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human VEGF both *in vitro* and *in vivo*. Bevacizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF. Unlike the antibodies that are targeted against EGFR, bevacizumab is directed against a soluble growth factor. Consequently, the antibody-antigen complex would not necessarily be expected to be internalized and degraded but would likely be predominantly cleared via hepatic endothelial cells with salvage of the antibody by FcRn. Bevacizumab would therefore primarily be catabolized.

There is limited PK and PD data published on bevacizumab. Such information is largely from the package insert for this mAb [71]. Based on a population PK analysis of 491 patients who received 1- to 20-mg/kg weekly doses, every 2 weeks or 3 weeks, the estimated half-life of bevacizumab was approximately 20 days (half-life range was 11 to 50 days). This is consistent with the expected pharmacokinetic behavior described earlier in hepatic endothelial cells. The clearance of bevacizumab was found to be dependent on body weight, gender, and tumor burden. Interestingly, patients with high tumor burden had a higher clearance.

Conclusions

The PK and PD of therapeutic mAbs are quite complex, being dependent on both the structure of the antibody and the specific antigen target. In very broad terms, antibodies that are directed against cell-surface antigens often exhibit nonlinear PK behavior while antibodies directed against

soluble receptors often exhibit linear behavior. However, there are numerous influences that need to be considered, including receptor shedding, the patient disease state and the physiology of the system being targeted.

Regardless of the PK behavior, the PD of most antibodies appear to be best described using indirect response type models. These models account for lag periods between drug administration and measurable response and also permit a persistent response when the antibody is completely cleared. Conducting PD evaluations requires a clear understanding of the interaction of antibodies with the immune system, and such models will continue to facilitate the evaluation of new therapeutic antibody candidates.

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